A NEW MICROSCOPIC METHOD FOR MEASURING THE VISCOSITY OF A LIQUID.

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Although the methods and apparatus for measuring the viscosity of a liquid found in literature are numerous in number and differ in principle, we can classify them into following categories:

(1) Those based on the measurement of the velocity of the liquid efflux from a horizontal or a vertical capillary tube.

(2) Those based on the measurement of the motion of a spherical or a cylindrical body in the liquid.

(3) Those based on the measurement of the angle of rotation of one cylinder dipped co-aixally in another hollow cylinder filled with the liquid.

(4) Those based on the measurement of the rotatory oscillation of a sphere, a cylinder, or a circular disk in the liquid.

(5) Other special methods.[1]

The new method of measurement proposed here differs in principle from those cited above and, having several characteristic features, is very useful, especially for the measurement of viscosity of viscous liquids, e.g. colloidal solutions of high concentration.

Theoretical.

The principle of this new method of measurement is quite simple. The liquid to be measured is made to flow under a definite pressure in a capillary tube of definite radius and length. The velocity of flow on the axis of the capillary tube is measured directly under a microscope and thus the absolute viscosity is determined. The theoretical formula is derived in the same way as in the case of Poisuille's law. When a

(1) A. Lampel, *Sitzb. Akad. Wiss. Wien*, (2a), **93** (1886), 291. H. Helmholtz and G. Piotrwski, *Sitzb. Akad. Wiss. Wien*, (2a), **40** (1868), 607. R. Ladenburg, *Ann. Physik*, (4), **27** (1908), 157. K. Mützel, *Wied. Ann.*, **43** (1891), 15. Subrahmaniam. *Indian J. Physics*, **1** (1926), 267. G. Stokes, *Phil. Mag.*, (4), **1** (1882), 337. F. Watson, *Phys. Rev.*, **15** (1902), 20. C. E. Guye and S. Mintz, *Arch. sci. phys. nat.*, (4), **26** (1908), 136. Gümbel, *Z. tech. Physik*, **1** (1920), 72. R. O. Boswall, *Phil. Mag.*, (7), **3** (1927), 994. G. Richardson, *J. Scient. Instrument*, **6** (1927), 337. H. A. Wilson, *Proc. Cambridge Phil. Soc.*, **10** (1900), 25. R. Z. Fürth, *Z. Physik*, **60** (1903), 313.

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liquid flows without slip or turbulence in a capillary tube, the following relation is given:

$$v = \frac{P}{4l\eta}(R^2-r^2) \qquad (1),$$

in which, v is the velocity of the flow at the distance r from the axis, l the length, and R the radius, of the capillary tube, P the difference of pressure at the two ends of the capillary tube, and η the viscosity of the liquid. Putting $r = 0$, we have v_0, the velocity on the axis of the tube:

$$v_0 = \frac{PR^2}{4l\eta} \qquad (2), \qquad \text{hence} \qquad \eta = \frac{PR^2}{4lv_0} \qquad (3).$$

As P, R, and l are known, we can determine the value η, if we can measure v_0. In practice, the measurement of P, R, and l is so simple that no explanation will be needed. It would be necessary to discuss how we can measure v_0, or whether v_0 is a measurable quantity.

I. v_0, a Measurable Value. Let us assume the viscosity of the liquid to be 10 (in absolute unit), the order of the viscosity of glycerine at ordinary temperature, and also, the radius of the capillary tube to be 0.05 cm. and its length 10 cm. Putting these values in the equation (2), we have $v_0 = 0.000625P$. Suppose a pressure is given by a water column, and let the height of the column be h, and we have $v_0 = 0.0613h$. The relations between v_0 and h are shown in Table 1. From this Table, we see that, under the pressure of the water column ranging from 1/100 mm. to 100 cm. in height, the velocity may be measured with the aid of a microscope and an ocular micrometer. For, as the displacement in the microscopic field is v_0t (t being the time required for the displacement), if we assume t=1000 sec., the measurement of the minimum value of v_0 in the table is possible. For a larger value of v_0, it is possible to measure with the same accuracy up to the value ca. h=0.1, if we take t smaller than above. In short, it may be said that, as v_0 is a measurable value even when radius R is 0.05 cm., we can always bring the value v_0 within the possible range of measurement by regulating the radius, the length,

Table 1.

h (cm.)	v (cm./sec.)
0.001	0.0000613
0.01	0.000613
0.1	0.00613
1	0.0613
10	0.613
100	6.13

and the pressure. So, it may be concluded that v_0 is a value measurable in the microscopic method.

II. The Range η can be Measured. Putting reasonable values for P,R,l in equation (3) let us try to determine the range where the measurement of η is possible.

R	(minimum value) 0.01 cm.	(maximum value) 0.5 cm.
l	5 cm.	20 cm.
P	1 cm. (water column)	150 cm. (mercury column)
v_0	0.00005 cm./sec.	0.005 cm./sec.

Measuring under these conditions with the aid of a stop watch, we have, within the accuracy of 0.001, the following numbers for η:

minimum value 2.4×10^{-1} (absolute unit), maximum value 5.0×10^{3} (absolute unit).

Therefore, the range is 10^{9}. For trial, if we calculate the range measurable with an Ostwald type viscosimeter, under the similar conditions, assuming the volume of liquid to be 5 c.c., and the time of flow 50 sec. (minimum), and 1000 sec. (maximum), we have:

minimum value 3.9×10^{-6} (absolute unit), maximum value 1.9×10^{6} (absolute unit).

In the method of falling ball, let the conditions be:

radius of the ball	(minimum value) 0.1 cm.	(maximum value) 0.5 cm.
time of fall	100 sec.	1000 sec.
density of ball	1.06	16.6

Furthermore, let the density of the liquid be 1 and the distance of the fall 10 cm., and we have, with the aid of Ladenburg's equation of correction:

minimum value 1.4×10^{0} (absolute unit), maximum value 6.5×10^{6} (absolute unit).

In practice, it is difficult to get perfectly spherical balls with different densities. Therefore, if we use the steel balls for ball-bearing on the market, we have the following numbers:

minimum value 16×10^{2} (absolute unit), maximum value 2.8×10^{6} (absolute unit).

Comparing these results, we see that the ordinary capillary viscosimeter is suitable for liquids of smaller viscosity, while the microscopic method is more useful for liquids of higher viscosity, and that the possible range of measurement is the largest in the latter.

III. Practical Method of Measuring v_0. A practical method of measurement in this microscopic method is as follows: first, fine solid particles are mixed with, and uniformly dispersed in, the liquid, and the liquid is put in a capillary tube. Then, with the aid of a microscope, we select any one particle among the particles which lie on the axis of the capillary tube. With this particle for an indicator, the velocity of the translation of the liquid is determined with the aid of an ocular micrometer and a stop watch. Therefore, it is necessary that the properties, the quantity to be added, and the radii of these particles should be examined.

(a) *Selection of Particles.* It is necessary that we should use the particles of a solid substance which is perfectly free from chemical reaction with, or from being dissolved by, the liquid to be investigated. For example, powder of carbon, platinum, silica, glass, Al_2O_3, SiC, etc. are suitable for this purpose. Though adsorption by the particles may be supposed to occur, this is negligible as the quantity to be added is very small. Of course, there is no need of taking this into consideration when the sample liquid is pure. It may be added that the particles must be free from swelling in the liquid.

(b) *Quantity of Particles to be Added.* The effect of the particles added on the viscosity of the liquid is as follows. Suppose that η, the viscosity of the sample, changes to η', on account of the addition of the particles, and that the measurements are done with the same capillary tube and under the same pressure, we have, by the equation (3),

$$\eta = \frac{R^2}{4lv_0} \quad (4), \qquad \eta' = \frac{R^2}{4lv_0'} \quad (5).$$

As the relation given by Einstein is applicable between the viscosity of the dispersed system and that of the medium, we have:

$$\eta' = \eta(1+2.5\varphi),$$

where φ is the value of the total volume the particles occupy, divided by the whole volume of the dispersed system. From (4), (5), and (6),

$$v_0 = (1+2.5\varphi)v_0', \qquad \text{hence} \qquad v_0' = v_0\left(\frac{1}{1+2.5\varphi}\right);$$

therefore, $v_0' = v_0(1-2.5\varphi)$, $\quad \frac{v_0'-v}{v_0} = -2.5\varphi$, $\quad \frac{\Delta v_0}{v_0} = -2.5\varphi$.

Let m be the quantity of the fine particles in 100 c.c. of the dispersed system, and ρ the density of the particle, then

$$\frac{\Delta v_0}{v_0} = -0.025\frac{m}{\rho}.$$

Assuming $m = 0.01$ g., we have Table 2.

Table 2.

ρ	$\Delta v_0/v_0$
0.5	0.0005
1	0.00025
2	0.00013
3	0.00008
4	0.00006
5	0.00005

As has already been noted, if the measurements of v_0 are done, in the case of $m = 0.01$ g., in the accuracy of 1/1000, all values of Table 2 becomes already less than the accuracy. Therefore, the velocity of the particle may be taken as the velocity of the liquid when the latter contains no particle. If we assume $\rho = 3$, the value $m = 0.1$ g. become within the range of the experimental errors. So, the use of the particle up to this value may well be admitted.

(c) *Radius and Number of the Particles.* The particle must be large enough to be an objective in the microscopic field, and the number of the particles must be sufficient so that the selection of the particle on the axis of the capillary tube may be done with ease. If we assume that one cubic millimetre of liquid contains 100 particles, the particle must be present in the probability of 10 particles on one millimetre of the axis. In order to obtain distribution of this density the radius of the particle ought to be

$$r = \sqrt[3]{\frac{3}{4}\frac{m}{\pi\rho n}}$$

where n is the number of particles in 1 cm.^{3}; r radius of the particle, m total mass of the particles, and ρ the density. Let $m = 0.001$ g., $\rho = 1$, $n = 10$, then we have $r = 0.0029$ cm. Therefore, it is sufficient, if we add the particles of radius of 0.03 mm. at the rate of 0.01 g. per 100 c.c. Moreover, this will also satisfy the conditions of the paragraph (b). In practice, as the selection of the objective particle can be done in the state of flow, the number of the particles may be far less than the rate just given.

(d) *Distance of Sedimentation Occurring during the Time of Measurement.* It may happen that, the particle, as the result of sedimentation due to gravity, escapes away from the focus of the microscope while being observed. This point may here be discussed. The distance

of sedimentation of a sphere, whose radius is r and density ρ_2, in a liquid with viscosity η and density ρ_1, will be given by the Stokes' law:

$$x = \frac{2}{9}\frac{r^2}{\eta}(\rho_2-\rho_1)gt\ .$$

If we assume the particle to be that of carborundum in water, then the distance x will be equal to 0.014 cm. for the time interval of $t = 1000$ sec.; and if $r = 0.0001$ cm., we have $x = 0.0003$ cm. Therefore, in this case, it is necessary to use the particle of $r = 0.0001$ cm., that is, 1 μ in radius. If the liquid has the density of the degree of glycerine, the particle may be of the radius of about 0.01 cm. With the liquid of higher viscosity, it is almost unnecessary to take this matter into consideration. From these investigations, it may be concluded that the microscopic method of measuring the viscosity of a liquid is a proposition not only practical, but also very convenient. Before describing the results of measurement, some distinctive characteristics of this method may be enumerated here:

(1) The measurement can be secured with a small quantity of the sample, only a few c.c. being sufficient.

(2) The possible range of measurement is wide.

(3) The measurement of the density of the sample is not necessary. In general, it is very difficult to measure the density of a viscous fluid. From this point of view, the present method may be said to be very useful.

(4) No special apparatus of measurement is required.

(5) As the presence of anomalous viscosity or a turbulent flow is recognized directly, the normal value of viscosity may be obtained.

Experimental.

I. Apparatus for Measurement. The measuring cell is made of glass with a horizontal capillary of ca. 10 cm. length and a vertical side tube of ca. 5 cm. height (Fig. 1). The schematic figure of the whole arrangement of the apparatus is given in Fig. 2. In the figure, AB is the measuring cell, M a microscope furnished with an ocular micrometer, F a green light filter which is used to get a sharp image of the objective particle in the field, S an incandescent electric lamp of Leitz make, the light source used in microphotography. At middle C of the capillary tube, two small pieces of glass plates are attached with balsam one above

and one below. They serve the purpose of keeping the rays from the light source parallel and of securing a sharp outline of the objective particle. D is a three-way stop cock, N a liquid manometer, P and Q

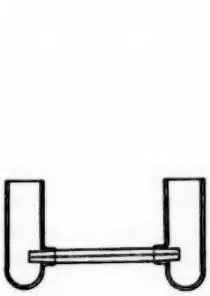

Fig. 1.

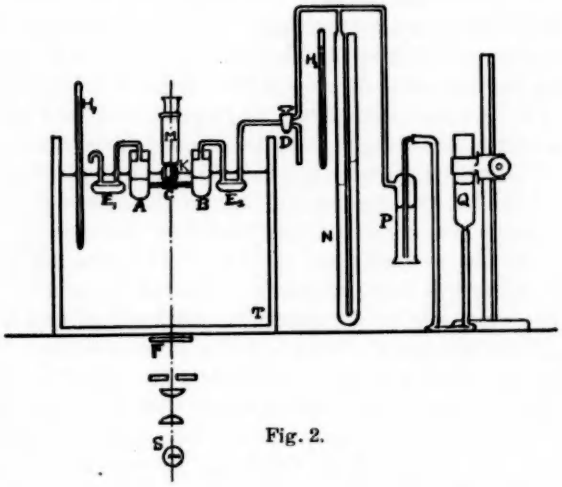

Fig. 2.

water holders which serve to produce pressure, P being fixed, Q movable up and down by a microscrew. E_1 and E_2 are small glass bulbs containing the same liquid as the sample, and serve to prevent the latter from evaporation or concentration, or taking moisture. In the experiments (1) and (2), where glycerine was used as the material for test, the measuring cell was kept at room temperature, but in the experiment (3), the cell was dipped in a thermostat, and the objective of the microscope was covered with a small glass cap K with a plane bottom, and was dipped in the liquid of the thermostat. The liquid used in N, P, and Q, was pure water, but, if necessary, mercury might be used.

II. Procedure. First, the tube for measurement AB is filled with the sample uniformly dispersed with objective particles and then all the connections are completed, as shown in Fig. 2. Then the selection of the objective particle in microscopic field is done. For this purpose, the axis of the capillary tube must be determined in the microscopic field. In order to do this, the ocular micrometer is fixed at right angles to the capillary tube. Then by the image of the walls of tube, the position of the axis of the tube is determined on the scale of the micrometer. Now

the ocular is turned, the microscope being fixed, just 90° and the scale of the micrometer is made to coincide with the axis of the tube. Then by raising and lowering the microscope vertically with the focus on the upper and then on the lower limit of the inner wall of the tube, two readings of the microscrew are obtained. The microscope is then fixed at the point just in the middle of these readings. Thus, the focus of the microscope is fixed on the axis of the capillary tube. In this position, the particle seen sharply in the centre of the field may be regarded to be on the axis of the tube. Now opening D, and making the liquid to flow in the capillary tube, a suitable particle is waited for to appear. When the objective particle is selected, the time is measure, with a stop watch, needed for the passage of the particle between two fixed points on the scale of the micrometer. Then the readings of the manometer as well as the thermometer are recorded. It is necessary to repeat the measurement several times under the same pressure until the maximum value of the velocity of the particle is obtained. Then the pressure is changed and measurement is done in the same manner. The measurement must also be done with the flow in the reverse way. The reason for this is that the pressure which causes the liquid to flow is not merely the pressure which is read on the manometer. Besides this, there is also a pressure, which affects the flow, due to the difference of the levels of the liquid in the two side tubes. In many cases, the levels in the side tubes are not equal in height under the condition with the stop-cock D closed. Especially, when the viscosity of the sample is very high, it is difficult that the levels in the two side tubes should become equal in height; and moreover when the surface of the liquid are in a curved state as it may well be, a considerable time will be required for it to regain the normal meniscus. It is necessary, therefore, to determine the value of the pressure due to this difference of the levels. Let us assume that under the positive or negative values of the pressure indicated on the manometer P_1, P_2, P_3, ... P_n, the velocities of flow, v_{01}, v_{02}, v_{03}, ... v_{0n}, have been obtained respectively. Plotting the diagram with P and v_0 as the coordinate axis, we get straight lines. In general, these straight lines do not pass through the origin. The distance, from the point where the line crosses the P-axis to the origin, indicates exactly the value of the pressure due to the difference of the levels. It may be assumed that this value is practically constant during measurements. A simple calculation shows this. Let the inner radius of the capillary tube be 0.05 cm., that of the side tube be 1.5 cm., and the number of the times of measurement be 10, the time needed for each measurements, the liquid passes only in one direction, and the volume of the liquid translated will be

$V = 1.47 \times 10^{-2}$ cm.^{3}, as $V = \dfrac{\pi R^4 P t}{8 l \eta}$, $R = 0.05$ cm., $P = 10^3$ dyne/cm., $l = 10$ cm., $\eta = 10$, and $t = 600$ sec. Therefore, the change of the levels in the side tubes will be $\Delta h = 2.00 \times 10^{-3}$ cm. which is within the error of the readings of the manometer. In practice, as the liquid travels in both directions, this value becomes smaller; and the pressure due to the difference of levels in the side tubes may be treated as unchanged during measurements. Now, the relation between P and v_0 becomes linear. (Of course, as is indicated in equation (2), it must be so theoretically.) The value P must be the algebraic sum of the two pressures, that is, $P = P_1 + P_2$, where P_1 is the value indicated in manometer, and P_2 is the value due to the difference of the levels. As regards the selection of the signs of P_1 and P_2, the pressures which make the objective particle move in the same direction have been given the same sign.

Now, we try to derive an equation by which the calculation can be worked out with the experimental data. Taking a general equation of a straight line, $P = a + bv_0$, where a corresponds to P_2 and may be put as constant and b is also a constant which must be determined from the experimental data. With the method of least square, the equations of condition may be obtained:

$$\frac{\delta}{\delta a}(P-a-bv_0)^2 = 0\,, \qquad \frac{\delta}{\delta b}(P-a-bv_0)^2 = 0\,,$$

therefore $$P-a-bv_0 = 0\,, \qquad Pv_0-av_0-bv_0^2 = 0\,.$$

From n numbers of observations, we have:

$$\sum_{i=1}^{n} P_i - na - b\sum_{i=1}^{n} v_{0i} = 0\,, \qquad \sum_{i=1}^{n} P_i v_{0i} - a\sum_{i=1}^{n} v_{0i} - b\sum_{i=1}^{n} v_{0i}^2 = 0\,.$$

From these equations, we get b:

$$b = \frac{\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0i} - n\sum_{i=1}^{n} v_{0i}P_i}{\left(\sum_{i=1}^{n} v_{0i}\right)^2 - n\sum_{i=1}^{n} v_{0i}^2}\,.$$

Thus if we have b, the viscosity η will be calculated with the equation (3).

$$\eta = \frac{R^2}{4l}\,\frac{P}{v_0} = \frac{R^2(P-a)}{4lv_0} = \frac{R^2}{4l}\,b = \frac{R^2}{4l}\,\frac{\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0i} - n\sum_{i=1}^{n} P_i v_{0i}}{\left(\sum_{i=1}^{n} v_{0i}\right)^2 - n\sum_{i=1}^{n} v_{0i}^2}\,.$$

III. Experimental Materials and Conditions of Measurement. The liquids used for the experiment were glycerine, acetone solution of cellulose acetate, the former being of the highest grade of Price make. The density of the sample used in the experiments (1) and (2), was $D_4^{30°} = 1.2506$, and that in the experiment (3) was $D_4^{25°} = 1.2532$. For the acetone solution of cellulose acetate, "Cellit L, low viscosity" was used. A certain quantity of this substance was dissolved in pure acetone and then made free from solid matters with a centrifuge. Then, to determine the concentration, a certain quantity was drawn and dried up under reduced pressure and the residue was weighed. The concentration was 15.90%. This solution was used in the experiments (4), (5), (6), and (7).

For the objective particles, carbon and carborundum particles were used. The carbon particles had been made by burning benzene, then washing, defatting with CS_2, and heating at about 300°C. The diameter of the carbon particles was ca. 1/10 mm. to 1/100 mm. The carborundum powder used was that on the market for use in grinding. The powder was made to suspend in water and, after a while, the middle layer of the suspension was collected. The powder thus obtained had

Table 3.

Exp. No.	1	2	3	4	5	6	7
Sample liquid	Glycerine No. 1	do.	Glycerine No. 2	Acetone solution of cellulose acetate	do.	do.	do.
Capillary tube	Tube No. 1 R= 0.040 cm. l=9.96 cm.	do.	do.	do.	do.	Tube No. 2 R= 0.0545 cm. l=9.91 cm.	Tube No. 3 R= 0.0965 cm. l=9.94 cm.
Objective	Carbon particle	do.	do.	Carborundum particle	do.	do.	do.
Quantity of particles added	0.1 g. per 100 c.c.	do.	do.	0.2 g. per 100 c.c.	do.	do.	do.
Measuring temp.	At room temp.	do.	25.00°C. ±0.01	30.0°C. ±0.1	26.4°C. ±0.1	do.	do.
Room temp.	30.0°C.	do.	20.0°C.	25.0°C.	29.5°C.	29.5°C.	28.0°C.
Remark			In thermostat	In simple bath	do.	do.	do.
Table No.	4	5	6	7	8	9	10
p–v Diagram	Fig. 3	Fig. 4	Fig. 5	Fig. 6.	Fig. 7	Fig. 8	Fig. 9

the diameter of the order of 1/50 mm. to 1/500 mm. These particles were treated first with concentrated hydrochloric acid, then with dilute hydrochloric acid, separated from the liquid with a centrifuge, made alkaline with ammonia, and washed with pure water, the centrifuge always being resorted to separate the liquid. Other experimental conditions are tabulated in Table 3.

IV. Results of Measurement and Their Discussion. (1) *Effect of the Quantity of Particles on Viscosity.* The experiment relative to the point discussed in **Theoretical, III, (b),** that is, the effect of quantity of particles added on viscosity was carried out and the following results were obtained.

(i) With an ordinary capillary viscosimeter, and glycerine without the particles, the following numbers were obtained: temp. 25.00°C. ± 0.01 (in thermostat); time of flow, (1) 19 min. 34.1 sec., (2) 19 min. 35.7 sec., (3) 19 min. 35.9 sec., (4) 19 min. 35.5 sec., mean value 19 min. 35.3 sec.

(ii) In the same glycerine, the carbon particles were mixed at the rate of 0.1 g. in 100 c.c. of glycerine, and the time of flow was measured with the same viscosimeter. The following data were obtained: temp. 25.00°C. ± 0.01; time of flow (1) 19 min. 36.2 sec., (2) 19 min 33.9 sec., (3) 19 min. 35.0 sec., (4) 19 min. 37.7 sec., mean value 19 min. 35.7 sec.

The increase of the time of flow due to the addition of the particle was 0.4 sec. This is 0.04% against 1175.3 sec. of the time of flow. This shows that the effect is within the experimental error and is quite negligible.

(2) *The Result of Measurement of Viscosity by the Microscopic Method.* (i) *Measurement at Room Temperature.* This was a preliminary measurement, which was indicated as experiment (1) and (2), and the results are given in Table 4 and 5. As the mean value $\eta = 4.36$ (absolute unit) was obtained. With an Ostwald viscosimeter, using the same sample, we obtained $\eta = 4.54$ (absolute unit). This is the value obtained in the thermostat of 30°C. As accuracy was not maintained in point of temperature, the comparison of the data is, strictly speaking, unreasonable but the result shows that the present method of viscosity measurement is quite a practical one.

(ii) *Measurement in the Thermostat.* From the preliminary test, the practical value of the present method of measurement was ascertained. So, definitive measurements were carried out. To gain the accuracy possible, the measuring tube was put in the thermostat, and the mano-

meter was read with a measuring microscope up to the order of 0.01 mm. The sample was glycerine No. 2. The results are shown in Table 6 as exp. (3). The observed value was $\eta^{25^\circ} = 5.93_3$ (absolute unit).

The viscosity of the liquid relative to water was measured with the same sample, in the same thermostat with an ordinary capillary viscosimeter and with the aid of the value of absolute viscosity of water, $\eta^{25^\circ} = 5.93_2$ (absolute unit) was obtained. In this case, the time of flow of water was 24.8 sec. and that of the sample liquid was 13120 sec., the density was 1.2532, and as density and absolute viscosity of water, the values $D_{4^\circ}^{25^\circ} = 0.99707$ and $\eta_{H_2O}^{25^\circ} = 0.008941$ respectively were used in the calculation.

The comparison of these two values of viscosity shows that coincidence is quite satisfactory. But, the coincidence at the second decimal place seems to be by chance, as the error of time measurement is to appear at this decimal place. As regards other factors, the errors will appear at the fourth decimal place. Therefore, it is not difficult to increase the accuracy of measurement by increasing the time of measurement. From this definitive measurement, it may be accepted that this new method of measurement gives quite satisfactory results in point of accuracy.

(iii) *Measurement of Viscosity of Acetone Solution of Cellulose Acetate.* With this new method, a measurement of absolute viscosity of acetone solution of cellulose acetate was carried out. The results of measurement are given in Table 7. (Exp. No. 4)

(3) *The Relation between the Inner Radius of the Capillary Tube and the Value of Viscosity Measured.* When the inner radius of the capillary part of measuring tube changes, the relation comes in as the power of square of the radius by equation (3). To verify this relation, experiments were carried out, with the same liquid and with the measuring tubes of different radius in capillary parts. These are exp. (5), (6), (7), and the results are shown in Table 8–11.

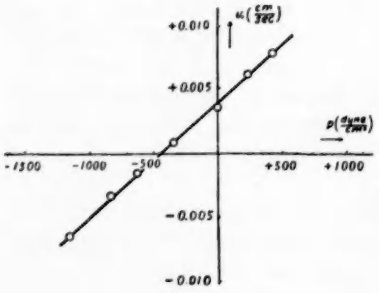

Fig. 3.

The sample was an acetone solution of cellulose acetate, same as stated in the preceding section. As the measurements were carried out in a simple bath, the results obtained are by no means perfect. However,

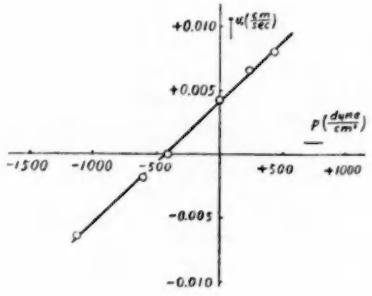

Fig. 4.

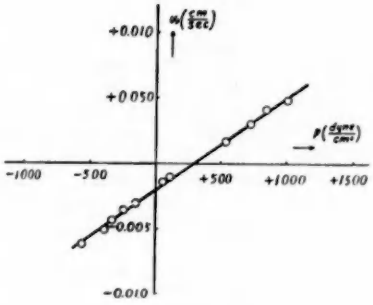

Fig. 5.

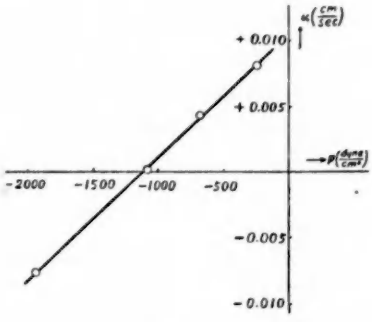

Fig. 6.

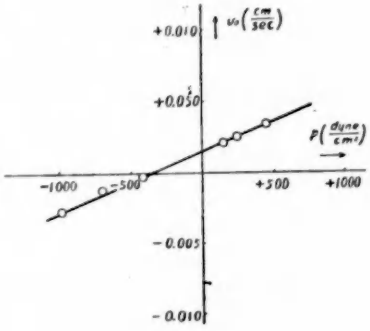

Fig. 7.

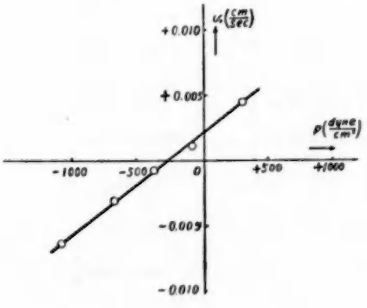

Fig. 8.

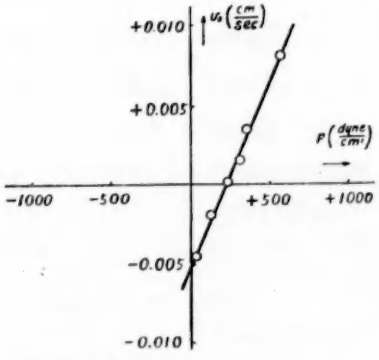

Fig. 9.

Table 4.

No.	$v_0\left(\frac{\text{cm.}}{\text{sec.}}\right)$	$P\left(\frac{\text{dyne}}{\text{cm.}^2}\right)$	v_0^2	Pv_0	Remark
1	−0.006413	−1133.0	0.00004113	+7.266	$\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0_i} = -15.73$
2	−0.001914	− 605.4	0.00000366	+1.159	$n\sum_{i=1}^{n} P_i\, v_{0_i} = +81.34$
3	−0.000161	− 410.1	0.00000003	+0.066	$\left(\sum_{i=1}^{n} v_{0_i}\right)^2 = +0.0001126$
4	+0.004275	± 0.000	0.00001828	±0.000	$n\sum_{i=1}^{n} v_{0_i}^2 = +0.001044$
5	+0.006678	+ 234.4	0.00004460	+1.565	$\frac{R^2}{4l} = 0.00004016$
6	+0.008144	+ 429.7	0.00006632	+3.500	$b = 1.043\times10^5$
$\sum_{i=1}^{n}$	+0.01061	−1484	+0.0001740	+13.56	$\eta = 4.18_7$
Viscosity (obs.)		1. By microscopic method			$\eta = 4.18_7$ (abs. unit)
		2. By capillary viscosimeter			$\eta = 4.54_4$ (abs. unit)

Table 5.

No.	$v^0\left(\frac{\text{cm.}}{\text{sec.}}\right)$	$P\left(\frac{\text{dyne}}{\text{cm.}^2}\right)$	v_0^2	Pv_0	Remark
1	+0.007895	+429.7	0.00006233	+3.393	$\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0_i} = -17.50$
2	+0.006256	+234.3	0.00003914	+1.466	$n\sum_{i=1}^{n} P_i\, v_{0_i} = +110.18$
3	+0.003620	±0.000	0.00001310	±0.000	$\left(\sum_{i=1}^{n} v_{0_i}\right)^2 = +0.00005664$
4	+0.000862	−351.5	0.00007430	−0.3030	$n\sum_{i=1}^{n} v_{0_i}^2 = +0.001187$
5	−0.001496	−624.9	0.00000224	+0.9348	$\frac{R^2}{4l} = 0.00004016$
6	−0.003268	−839.8	0.00001068	+2.732	$b = 1.130\times10^5$
7	−0.006413	− 1172	0.00004113	+7.516	$\eta = 4.53_8$
$\sum_{i=1}^{n}$	+0.007526	− 2324	0.0001695	+15.74	
Viscosity (obs.)		1. By microscopic method			$\eta = 4.53_8$ (abs. unit)
		2. By capillary viscosimeter			$\eta = 4.54_1$ (abs. unit)

Table 6.

No.	$v_0\left(\frac{\text{cm.}}{\text{sec.}}\right)$	$P\left(\frac{\text{dyne}}{\text{cm.}^2}\right)$	v_0^2	Pv_0	Remark
1	−0.001394	+59.71	0.00000194	−0.08384	$\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0_i} = -17.47$
2	−0.003000	−152.7	0.00000300	+0.4581	$n\sum_{i=1}^{n} P_i v_{0_i} = +214.35$
3	−0.003538	−243.7	0.00001252	+0.8623	$\left(\sum_{i=1}^{n} v_{0_i}\right)^2 = +0.0001184$
4	−0.004275	−331.8	0.00001828	+1.4185	$n\sum_{i=1}^{n} v_{0_i}^2 = +0.0016874$
5	−0.005130	−394.5	0.00002633	+2.0236	$\frac{R^2}{4l} = 0.00004016$
6	−0.006181	−560.9	0.00003820	+3.4666	$b = 1.478\times10^5$
7	−0.001016	+112.6	0.00000103	−0.1143	$\eta = 5.93_3$
8	+0.001676	+533.5	0.00000281	+0.8944	
9	+0.003054	+723.4	0.00000933	+2.2088	
10	+0.004171	+832.4	0.00001740	+3.4701	
11	+0.004750	+1028.0	0.00002256	+4.8820	
$\sum_{i=1}^{n}$	−0.010884	+1605.6	0.0001534	+19.486	
Viscosity (obs.)		1. By microscopic method			$\eta = 5.93_3$ (abs. unit)
		2. By capillary viscosimeter			$\eta = 5.93_2$ (abs. unit)

Table 7.

No.	$v_0\left(\frac{\text{cm.}}{\text{sec.}}\right)$	$P\left(\frac{\text{dyne}}{\text{cm.}^2}\right)$	v_0^2	Pv_0	Remark
1	+0.008143	−254.2	0.00006631	−2.070	$\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0_i} = -7.919$
2	+0.004385	−684.5	0.00001928	−3.001	$n\sum_{i=1}^{n} P_i v_{0_i} = +76.80$
3	+0.0005109	− 1085	0.00000026	−0.5543	$\left(\sum_{i=1}^{n} v_{0_i}\right)^2 = +0.000002048$
4	−0.007600	− 1946	0.00005776	+14.79	$n\sum_{i=1}^{n} v_{0_i}^2 = +0.0007980$
5	−0.004008	− 1564	0.00001606	+6.196	$\frac{R^2}{4l} = 0.00004016$
$\sum_{i=1}^{n}$	+0.001431	− 5534	0.0001596	+15.36	$b = 1.064\times10^5$
Viscosity (obs.)		$\eta = 4.27_4$ (abs. unit)			

Table 8.

No.	$v_0\left(\frac{\text{cm.}}{\text{sec.}}\right)$	$P\left(\frac{\text{dyne}}{\text{cm.}^2}\right)$	v_0^2	Pv_0	Remark
1	+0.003466	+449.3	0.00001201	+1.5573	$\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0_i} = +32.70$
2	+0.002559	+253.9	0.00000256	+0.6497	$n\sum_{i=1}^{n} P_i v_{0_i} = +295.60$
3	+0.002129	+156.3	0.00000453	+0.3328	$\left(\sum_{i=1}^{n} v_{0_i}\right)^2 = +0.00005663$
4	−0.0002392	−420.0	0.00000006	+0.1005	$n\sum_{i=1}^{n} v_{0_i}^2 = +0.00151$
5	−0.001207	−713.0	0.00000146	+0.8606	$\frac{K^2}{4l} = 0.00004016$
6	−0.002788	−986.5	0.00000777	+2.7504	$b = 2.403\times10^5$
7	−0.01166	−3086	0.00013596	+35.983	
$\sum_{i=1}^{n}$	−0.007525	−4346	0.00016435	+42.23	
Viscosity (obs.)	$\eta = 9.65_4$ (abs. unit)				

Table 9.

No.	$v_0\left(\frac{\text{cm.}}{\text{sec.}}\right)$	$P\left(\frac{\text{dyne}}{\text{cm.}^2}\right)$	v_0^2	Pv_0	Remark
1	−0.006334	−1104.0	0.00004012	+6.993	$\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0_i} = +8.765$
2	−0.003109	−693.5	0.00000967	+2.156	$n\sum_{i=1}^{n} P_i v_{0_i} = +52.10$
3	−0.0006503	−390.7	0.00000042	+0.2541	$\left(\sum_{i=1}^{n} v_{0_i}\right)^2 = +0.00001934$
4	+0.001196	−97.67	0.00000143	−0.1168	$n\sum_{i=1}^{n} v_{0_i}^2 = +0.0003595$
5	+0.004500	+293.0	0.00002025	+1.1318	$\frac{K^2}{4l} = 0.00007593$
$\sum_{i=1}^{n}$	−0.004398	−1992.9	0.00007189	+10.42	$b = 1.274\times10^5$
Viscosity (obs.)	$\eta = 9.67_6$ (abs. unit)				

Table 10.

No.	$v_0\left(\frac{\text{cm.}}{\text{sec.}}\right)$	$P\left(\frac{\text{dyne}}{\text{cm.}^2}\right)$	v_0^2	Pv_0	Remark
1	−0.004499	+29.31	0.00002024	−0.1319	$\sum_{i=1}^{n} P_i \sum_{i=1}^{n} v_{0_i} = +11.036$
2	−0.001936	+136.8	0.00000375	−0.2649	$n\sum_{i=1}^{n} P_i v_{0_i} = +35.598$
3	+0.0001494	+234.5	0.00000002	+0.03503	$\left(\sum_{i=1}^{n} v_{0_i}\right)^2 = +0.00004629$
4	+0.001560	+302.9	0.00000243	+0.4725	$n\sum_{i=1}^{n} v_{0_i}^2 = +0.0006186$
5	+0.003514	+342.0	0.00001235	+1.202	$\frac{R^2}{4l} = 0.0002342$
6	+0.008016	+576.4	0.00006426	+4.620	$b = 4.222\times10^4$
$\frac{1}{n}\sum_{i=1}^{n}$	+0.006804	+1622	0.0001031	+5.933	
Viscosity (obs.)		$\eta = 9.88_7$ (abs. unit)			

Table 11.

Tube No.	l (Length of tube)	R (Radius of tube)	R^2/l	b	$K\left(=\frac{R^2}{l}\times b\right)$
1	9.96	0.0400	0.0001606	2.403×10^5	38.6
2	9.91	0.0545	0.0003037	1.274×10^5	38.7
3	9.94	0.0965	0.0009368	4.222×10^4	39.6

as is shown in Table 11, the products of R^2/l and b are almost constant for all capillary tubes. This is what the theory requires. These results also indicate that the microscopic method of measurement is practical.

Summary.

(1) In connection with the method for measuring the viscosity of a liquid, a microscopic method, quite different from the methods hitherto known has been proposed and its possibility has been examined.

(2) The advantages of this microscopic method has been enumerated.

(3) The apparatus and the procedure of the measurement has been described.

(4) The theoretical relation of the influence of the fine particles added to the sample liquid on the viscosity of the liquid has been confirmed by experiment.

(5) The viscosity of glycerine and acetone solution of cellulose acetate has been measured by this new method.

(6) The results of measurements by the new method and those by the Ostwald viscosimeter have been compared, and the accuracy of the new method has been shown.

(7) It is shown that the relation between the viscosity and the radius of the measuring capillary tube is in accord with the theory.

The author desires to express here his feeling of obligation to Professor S. Horiba for his kind guidance throughout the investigation. The author also desires to express his gratitude to the Department of Education which supplied him with the necessary expenses for the investigation by granting him a Subsidy for Encouragement of the Study of Natural Science. The author is also indebted to Mr. T. Daimon who assisted him in the experiments.

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BIOCHEMICAL STUDIES ON "SOTETSU" (*Cycas revoluta* Thunb.). IV. ON SOTETSU-EMULSIN.

By **Kôtaro NISHIDA.**

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Dr. K. Yoshimura[1] in our college discovered that "Sotetsu," especially its seed, contains a fair amount of formaldehyde, and stated that the sotetsu-poison is due to the presence of the formaldehyde. He prepared a distillate from the alcoholic extract of mashed sotetsu-seed, in which he detected formaldehyde by using Nessler's reagent and ammoniacal silver nitrate solution, and then identified by preparing hexamethylenetetramine. His study greatly arouses our interest, especially from the biochemical point of view; he did not, however, investigate the form of formaldehyde in "Sotetsu."

(1) K. Yoshimura, *Bull. Kagoshima Agr. College*, **5** (1922), 25.

The writer[2] at first confirmed Yoshimura's experimental results by a different method, Rimini's and Vitali's colour reactions, and by preparing formaldomedon for identification. And then the writer found that the formaldehyde in "Sotetsu" is in the combined state of a new glucoside, and liberated it from the glucoside by the action of the emulsin in "Sotetsu."

So the writer intended to study the new glucoside containing formaldehyde and the sotetsu-emulsin. In the present paper, mainly some properties of sotetsu-emulsin are described.

I. Identification of Formaldehyde in Sotetsu-seed. Twenty hulled sotetsu-seeds were mashed and extracted with boiling 90% alcohol, and the alcoholic extract was evaporated to a small volume under reduced pressure. The distillate of the steam distillation of the above solution gave intensive Rimini's and Vitali's reactions, and 0.8 g. of its domedon crystallized out on adding 1.0 g. dimedon. The domedon thus obtained formed lustrous colourless prisms and melted at 188°C. (uncorr.), agreeing with formaldomedon.

II. The Form of Formaldehyde in Sotetsu-seed. Experiment A. A hulled sotetsu-seed was mashed and put into the thermostat at 35°C., and after 4 hours it was steam-distilled. The distillate gave intensive colour reactions for formaldehyde. Sulphuric acid was added to the residue of steam distillation and the distillation was continued further; in the second distillate, the formaldehyde reaction was inferior. **Experiment B.** A carefully hulled sotetsu-seed was boiled for 30 minutes under a reflux condenser, and treated in the same way as above. In this case, even in the first distillate the reactions of formaldehyde were very weak.

The results of quantitative estimation of formaldehyde are shown in Table 1.

Table 1.

Experiment	Sample (g.)	HCHO mg. in 1st distillate	HCHO mg. dissolved in boiling water
A	9.3374	13.3	—
B	8.4887	1.3	5.3

Thus, the formaldehyde in sotetsu-seed is an enzymatic decomposition product from its compound.

(2) K. Nishida, *J. Agr. Chem. Soc. Japan*, **11** (1935), 357.

III. Preparation of Sotetsu-emulsin. The hulled sotetsu-seeds were mashed and mixed thoroughly with an equal weight of water containing a small amount of toluene, and put into an ice-box. After one night, the mass was pressed on a cloth, and the filtered liquid was acidified with acetic acid to precipitate proteins. From the filtrate, the crude enzyme was precipitated with alcohol or acetone. The precipitate was washed with absolute alcohol or acetone, then with ether and finally it was dried in vacuo over sulphuric acid. For the further purification the resulting precipitate was redissolved in water and reprecipitated with alcohol or acetone and treated similarly to the above operation. By using acetone as precipitant, the yield of the emulsin-preparation from 4 kg. sample was 6.0 g.

IV. Usual Methods of Estimation of Enzyme Activity. The digestion mixtures usually consisted of 15 c.c. of 1% salicin solution, 5 c.c. of 0.4–0.8% sotetsu-emulsin solution, and 2 c.c. of Sörensen's buffer solution. The mixtures, preserved with toluene, were kept at 37°, usually for digestion hours of 90 minutes. Enzyme solution, inactivated by heat, were employed as controls in usual way. The enzyme activity has been measured by determining the reducing action of the decomposed salicin, e.g. with respect to Fehling's solution according to Bertrand.

V. Optimum *p*H of the Sotetsu-emulsin. In determining the *p*H optimum, citrate-HCl or phosphate buffer was used. The activity of

Table 2.

Salicin Hydrolysis at Various Hydrogen Ion Concentrations.

(15 c.c. of 1% salicin, 5 c.c. of 0.4% enzyme, 37°, 90 mm.)

*p*H	Standard $KMnO_4$ (c.c.)	Salicin hydrolysed (%)	$\frac{10^5}{t}\ln\frac{a}{a-x}$	$\frac{\varphi}{\Phi}$
3.6	3.10	16.8	2.04	0.52
4.3	5.05	27.7	3.60	0.92
4.5	5.15	28.3	3.70	0.95
4.8	5.35	29.5	3.88	0.99
5.1	5.40	29.7	3.91	1.00
5.2	5.20	28.6	3.74	0.96
5.8	4.90	26.9	3.48	0.89
5.9	4.80	26.3	3.39	0.87
6.1	4.55	24.8	3.17	0.81
7.0	2.45	13.2	1.57	0.40

sotetsu-emulsin at various values of *p*H are summarized in Table 2, in which the *p*H value indicates the mean of the initial and final reactions.

Thus the sotetsu-emulsin exhibited optimum activity for salicin hydrolysis, in the solution of *p*H 5.1. E. Vulquin[3] stated that almond-emulsin had an optimum activity at *p*H 4.4; whereas *p*H 4.7 to 5.1 for β-methylglucoside, 6.0 for amygdalin, and 4.2 for lactose were optimum for almond-emulsin according to Willstätter and Csányi.[4]

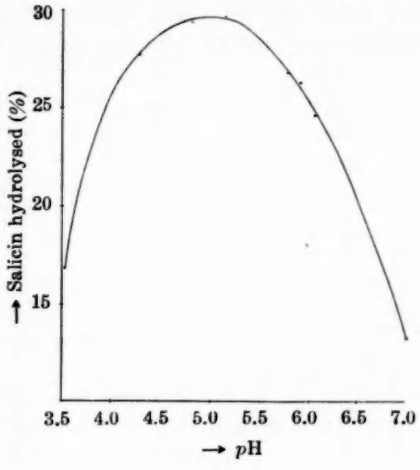

Fig. 1.

VI. Optimum Temperature of the Sotetsu-emulsin. The optimum temperature of sotetsu-emulsin was sought by using the usual mixtures of *p*H 4.8. Each of these mixtures was immersed in a water bath at different temperatures for 90 minutes. The rate of decomposition of salicin by sotetsu-emulsin at various temperatures was estimated as shown in Table 3.

Table 3.

Optimum Temperature for Salicin Hydrolysis by Sotetsu-emulsin.

(15 c.c. of 1% salicin, 5 c.c. of 0.8% enzyme, *p*H 4.8, 90 min.)

Temperature (°C)	Standard $KMnO_4$ (c.c.)	Salicin hydrolysed (%)	$\frac{10^3}{t}\ln\frac{a}{a-x}$	$\frac{\varphi}{\Phi}$
30	8.95	50.8	7.88	0.44
35	9.70	55.4	8.97	0.50
40	10.35	58.9	9.88	0.55
45	11.15	64.5	11.50	0.64
50	12.85	75.3	15.53	0.87
55	13.55	80.0	17.88	1.00
60	13.45	79.3	17.40	0.97
65	10.15	58.2	9.69	0.54

(3) E. Vulquin, *Soc. biol., Paris*, **70** (1911), 270, 763.
(4) R. Willstätter and W. Csányi, *Z. physiol. Chem.*, **117** (1921), 172.

According to the above results, the reaction velocity of the sotetsu-emulsin is accelerated as the temperature is raised to 55°C. On further elevation of the temperature the reaction velocity begins to diminish, and when the *p*H value of the solution is 4.8, we see that the sotetsu-emulsin may act at a maximum activity at 55–60°C.

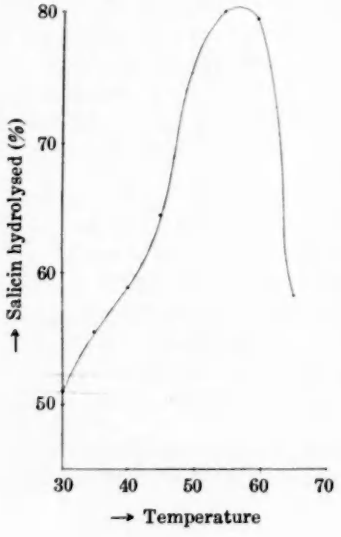

Fig. 2.

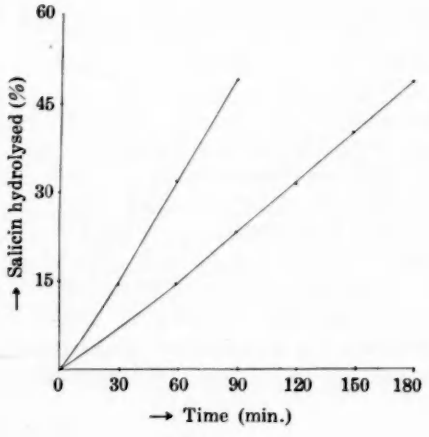

Fig. 3.

VII. Reaction Course of Sotetsu-emulsin. Hudson and Paine[5] studying the hydrolysis of salicin with almond-emulsin found that the reaction course of the enzyme follows the monomolecular reaction. J. Giaga[6] states that the reaction course of emulsin varies with the enzymes used and follows a sort of selective mass action law. Willstätter and Csányi[7] reported that the reaction of almond-emulsin for amygdalin hydrolysis was not found as a monomolecular reaction. And also according to Helferich's experiment,[8] in the reaction of Kahlbaum's emulsin-preparation for salicin hydrolysis, the monomolecular reaction constant increased in course of time. In our experiments with sotetsu-emulsin, as shown in table 4, we found almost the same tendency.

(5) Hudson and Paine, *J. Am. Chem. Soc.*, **31** (1909), 1242.
(6) J. Giaga, *J. chim. phys.*, **19** (1921), 77; *Chem. Abst.*, **16** (1922), 2336.
(7) *Loc. cit.*
(8) B. Helferich, *Z. physiol. Chem.*, **117** (1921), 159.

Table 4.

Kinetics of Salicin Hydrolysis by Sotetsu-emulsin.

(15 c.c. of 1% salicin, 5 c.c. of 0.4% enzyme, pH = 4.4, 37°)

Time (min.)	Standard $KMnO_4$ (c.c.)	Salicin hydrolysed (%)	$\frac{10^3}{t} \ln \frac{a}{a-x}$
60	2.70	14.2	2.55
90	4.30	22.9	2.89
120	5.80	31.4	3.14
150	7.25	39.7	3.37
180	8.80	48.9	3.73

VIII. Reaction Velocity and Enzyme Amount. According to Auld,[9] the reaction velocity of amygdalin hydrolysis by almond-emulsin is proportional to the concentration of the enzyme. Willstätter and Csányi[10] reported that the reaction velociy of hydrolysis of amygdalin, β-methyl-glucoside lactose and raffinose by enzyme is proportional to the amount of the enzyme. In our experiments, also on the decomposition of salicin the same relation was observed. The results are summarized in Table 5.

Table 5.

Reaction Velocity and Enzyme Amount. (pH = 4.4, 37°)

Substrate	Sotetsu-emulsin	Time (min.)	Standard $KMnO_4$ (c.c.)	Salicin hydrolysed (%)
15 c.c. of 1% Salicin	5 c.c. of 0.4% solution	60	2.70	14.2
,,	5 c.c. of 0.8% solution	30	2.70	14.2
,,	5 c.c. of 0.4% solution	120	5.80	31.3
,,	5 c.c. of 0.8% solution	60	5.85	31.7
,,	5 c.c. of 0.4% solution	180	8.80	48.9
,,	5 c.c. of 0.8% solution	90	8.80	48.9

IX. Total Hydrolysis of Salicin by Sotetsu-emulsin. Bertrand and Compton[11] reported that the salicin in concentration very much superior to that found in plants was completely hydrolysed by the action of emulsin. Also we found that glucose was completely splitted off from salicin, as shown in Table 6.

(9) S. J. M. Auld, *J. Chem. Soc.*, **93** (1908), 1251.

(10) *Loc. cit.*

(11) G. Bertrand and A. Compton, *Ann. Inst. Pasteur*, **39** (1925), 355; *Chem. Abst.*, **19** (1925), 2962.

Table 6.

Total Hydrolysis of Salicin by Sotetsu-emulsin.

(15 c.c. of 1% salicin, 5 c.c. of 0.8% emulsin, pH = 4.8, 37°)

Time (hour)	Standard $KMnO_4$ (c.c.)	Salicin hydrolysed (%)	$\frac{10^3}{t} \ln \frac{a}{a-x}$
5	15.70	94.5	9.67
7	16.15	97.5	8.79
8	16.50	100.0	9.59
15	16.50	100.0	—

Summary.

The principal experimental results described above are summarized as follows:

(1) Sotetsu-seed contains a glucoside of formaldehyde and this substance splits off formaldehyde by the action of sotetsu-emulsin.

(2) The emulsin was easily prepared from sotetsu-seed and some of its properties were studied in the case of salicin as substrate.

(3) The sotetsu-emulsin exhibited maximum activity in the solution of pH near 5.0.

(4) It was observed that the optimum temperature of sotetsu-emulsin for salicin hydrolysis is 55–60°C. near the optimum hydrogen ion concentration.

(5) In the reaction course of sotetsu-emulsin for salicin hydrolysis, the monomolecular reaction constant increased in course of time.

(6) The reaction velocity of salicin hydrolysis is proportional to the amount of sotetsu-emulsin.

(7) The salicin in 15 c.c. of 1% solution was completely hydrolysed by the action of sotetsu-emulsin in 5 c.c. of 0.8% solution, at the end of 8 hours in the digestion mixture of pH value 4.8 and temperature 37°C.

In conclusion, the writer wishes to express his thanks to Mr. A. Yamada for the kind assistance. He is also indebted to the Nippon Gakujutsu Shinkokwai, a part of the research expenses being defrayed from its grant.

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CATALYTIC HYDROGENATION OF ACETONE-COMPOUNDS OF α-HYDROXY-ACIDS.*

By Haruomi ÔEDA.

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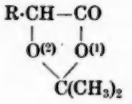
In a previous communication,[1] preparation of acetone-compounds of some α-hydroxy-acids was reported. As already described in that paper, the manipulation for their preparations is simple and their yields are fairly good in almost all cases and there is the prospect that they can be favourably used as a starting material for several reactions.

In the present communication studies on catalytic hydrogenation of these compounds under pressure are reported. Copper-chromium oxide was used as catalyzer, which had been proved by H. Adkins[2] to be very effective in the reduction of esters into alcohols.



In the heterocyclic ring of acetone-compounds of α-hydroxy-acids, one of the oxygen atoms (1) may functionate as ester, and on hydrogenation, it is probable that the cleavage of linking might occur in carbonyl side of the oxygen, as in the hydrogenation of esters:

$$R{\cdot}COOC_2H_5 \longrightarrow R{\cdot}CH_2OH + HOC_2H_5$$

Another oxygen (2) in the ring may be compared with those in ethers or acetals. Several papers[3] are published on the hydrogenation of acetals and in every instance cleavage of the molecule occurs in the aldehyde side of the oxygen with regeneration of the alcohol, detaching the aldehyde as a hydrocarbon:

$$\begin{matrix} C_2H_5O \\ C_2H_5O \end{matrix}\!\!\times\!\!\diamond\!\!\times CH{\cdot}CH_3 \longrightarrow 2\,C_2H_5OH + CH_3{\cdot}CH_3$$

Recently K. Yoshikawa[4] obtained hexahydric alcohols in the hydrogenation of many hexoses. It may also be regarded as the same cleavage

* Studies on Hydroxy-Acids and their Derivatives. III.

(1) H. Ôeda, this Bulletin, **10** (1935), 187.

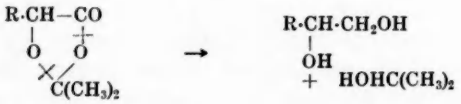
(2) H. Adkins, K. Folkers, *J. Am. Chem. Soc.*, **53** (1931), 1095; **54** (1932), 1145. H. Adkins, B. Wojcik, L. Covert, *ibid.*, **55** (1933), 1669.

(3) T. Kariyone, *J. Pharm. Soc. Japan*, **43** (1923), 51. M. Bergmann, N. Carter, *Z. physiol. Chem.*, **191** (1930), 211. L. Covert, R. Connor, H. Adkins, *J. Am. Chem. Soc.*, **54** (1932), 1651.

(4) K. Yoshikawa, *Bull. Inst. Phys. Chem. Research, Japan*, **13** (1934), 1045.

of acetal linking in the sugar molecules. These facts suggest that, through hydrogenation, cleavage in linking (2) may occur in isopropylidene side of the oxygen.

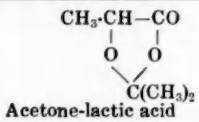
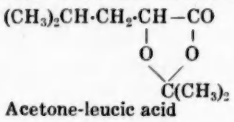
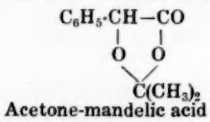
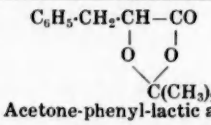
From the above consideration, the hydrogenation of these acetone-compounds may result in the formation of corresponding glycols as well as isopropyl alcohol.



The experimental results of the present study agree with the above expectation except in one case and they are summarized in Table 1.

H. Adkins has studied on the hydrogenation of ethyl lactate[5] and ethyl mandelate[6] in the presence of copper-chromium oxide and he found that the former yielded propylene glycol, while, it was not the case

Table 1.

Substance	Products
 Acetone-lactic acid	$CH_3 \cdot CHOH \cdot CH_2OH$ Propylene glycol + $HOHC(CH_3)_2$ Isopropyl alcohol —
 Acetone-leucic acid	$(CH_3)_2CH \cdot CH_2 \cdot CHOH \cdot CH_2OH$ Isohexylene glycol + $HOHC(CH_3)_2$ Isopropyl alcohol + $(CH_3)_2CH \cdot CH_2 \cdot CH_2 \cdot CH_2OH$ Isohexyl alcohol
 Acetone-mandelic acid	— + $HOHC(CH_3)_2$ Isopropyl alcohol + $C_6H_5 \cdot CH_2 \cdot CH_2OH$ β-Phenyl-ethyl alcohol + Aromatic hydrocarbon
 Acetone-phenyl-lactic acid	$C_6H_5CH_2 \cdot CHOH \cdot CH_2OH$ Benzyl-ethylene glycol + $HOHC(CH_3)_2$ Isopropyl alcohol + $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2OH$ γ-Phenyl-propyl alcohol + $C_6H_5 \cdot CH_2 \cdot CHOH \cdot CH_3$ Benzyl methyl carbinol

(5) K. Folkers, H. Adkins, *J. Am. Chem. Soc.*, **54** (1932), 1145.
(6) H. Adkins, B. Wojcik, L. Covert, *J. Am. Chem. Soc.*, **55** (1933), 1669.

with the latter, which never yielded glycol, but gave a considerable amount of ethyl-benzene together with phenyl-ethyl alcohol. He concluded that hydroxyl attached to a carbon having benzenoid ring was very labile and hydrogenation of further advanced stage occurred in this case.*

The results obtained in the present study are in good agreement with the works of Adkins as shown in Table 1. Hydrocarbon yielded from mandelic ester was mentioned by Adkins as ethyl-benzene. Corresponding fraction in distillation has been also obtained from the acetone-compound.**

Expected course of hydrogenation was observed in the other two acetone-compounds. Both of them were found to give corresponding glycol and monohydric alcohol or alcohols which may be regarded as products of further hydrogenation of the glycol.

A viscous odourless liquid boiling at 111–113.5° under 15 mm. was obtained from acetone-leucic acid and it was found to be isohexylene glycol by the analysis as well as by the formation of bis-phenylurethane (m.p. 115–116°). No information of this glycol is found in literature.

By the action of phenyl magnesium bromide upon α-monochlorohydrine of glycerol, Grignard[7] obtained a mixture of two isomeric glycols, which boils at 163–165° under 12 mm. Benzyl-ethylene glycol[8] yielded from acetone-phenyl-lactic acid corresponds to one of the glycols of Grignard. Its bis-phenylurethane was prepared, which was isolated in two forms with different melting points. Neither of them contains solvent of crystallisation. The form, which was obtained by crystallisation from alcohol, is crystalline and melts at 108–109°, while the other obtained from benzene solution is amorphous and melts at 132–133°. They are interconvertible by changing the solvent in crystallisation. Monotropic change was also observed on heating, that is, the urethane melting at 108–109° was gradually transformed into the other form on prolonged heating at 100–110°.

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* It was shown by Adkins that compounds of similar type respecting their hydroxyls such as benzyl alcohol, phenyl methyl carbinol, and diphenyl carbinol were readily hydrogenated with the same catalyzer to the corresponding hydrocarbons at 150 to 200°, temperature lower than that necessary for hydrogenation of esters.

** In the present study it could not be identified as ethyl-benzene, it yielded benzoic acid on oxidation and it corresponds to a mixture of toluene and ethyl-benzene with regards to its boiling point.

(7) V. Grignard, *Compt. rend.*, **141** (1905), 45; *Ann. chim. phys.*, (8), **10** (1907), 31.

(8) Recently this glycol was prepared by Hershberg, starting from allyl-benzene through the di-benzoic ester, *Helvetica Chim. Acta*, **17** (1934), 351.

Experimental Part.

Copper-chromium oxide catalyzer (containing a little barium) was prepared according to the description of Adkins[9] by the calcination of the precipitates formed by mixing ammoniacal solutions of copper and barium nitrates and of ammonium bichromate. Its composition is to be $2(0.9CuO + 0.1BaO) + Cr_2O_3$ when estimated from the proportion of reagents used.

Acetone-compound (prepared from hydroxy-acid and acetone by dehydration with conc. sulphuric acid) was mixed with 1/5–1/10 of its weight of the above catalyzer without any diluent. The hydrogenation was carried out at the temperature of 240–260° for 0.5–4.5 hours in an autoclave (capacity 250 c.c.) provided with shaking equipment, initial pressure of hydrogen being 100–120 atmospheres at room temperature.

Hydrogenated products were separated from catalyzer with glass filter and they were fractionated through Widmer column into several fractions under ordinary or reduced pressure.

Hydrogenation of Acetone-*dl*-lactic acid. Acetone-compound (b.p. 49.0–49.5° under 11 mm.) 30 g. (0.23 mol). Catalyzer 5.0 g. Time of hydrogenation 4.0 hours at 240–260°. Reaction product was fractionated into two parts.

I	79–81° under ordinary pressure	4.0 g.
II	82–85° under 11 mm.	10.2 g.

Fraction I was found to be isopropyl alcohol by converting it into naphthylurethane. *Naphthylurethane.* Mixture of the alcohol (1.0 g.) and α-naphthyl isocyanate (2.5 g.) was heated up to 150° (in glycerine bath) for one hour. On cooling, it turned to a hard cake, which was dissolved in hot ligroin and filtered. Naphthylurethane (0.8 g.) was obtained from the filtrate, which melted at 104–105° after repeated recrystallisation from ligroin (Table 2).

Fraction II distilled at 94–96° under 20 mm. after refractionation and it was found to be propylene glycol by converting it into bis-phenylurethane. *Bis-phenylurethane.* The glycol (1.0 g.) and phenyl isocyanate (2.0 g.) were mixed and heated at 120–140° for one hour. Five times of ligroin was added, the mixture well stirred, the ligroin was decanted off, and the remaining liquid was dissolved in benzene. The solution was evaporated to a small bulk, from which the crude urethane crystallized out (1.0 g., m.p. 120–135°). After repeated recrystallisation from ligroin and finally from alcohol it melted at 143–144° and showed no change of m.p. on further purification (Table 2).

Hydrogenation of Acetone-*l*-leucic acid. Acetone-compound (b.p. 84.5–85.5° under 13 mm.) 30 g. (0.175 mol). Catalyzer 4.0 g. Time of hydrogenation $4\frac{1}{3}$ hours at 240–260°. Fractionation (filtered solution, 24.6 g.):

I	80– 81° under ordinary pressure	7.6 g.
II	140–150° ,,	4.4 g.
III	56–105° under 15 mm.	1.1 g.
IV	110–116° ,,	9.3 g.

(9) R. Connor, K. Folkers, H. Adkins, *J. Am. Chem. Soc.*, **54** (1932), 1139. The above catalyzer is specified as No. 37 KAF in this paper.

Fraction II was treated with 10% NaOH to remove acetone-compound and the neutral part was collected by extracting with ether and the extract was refractionated. Main part (3.2 g.) boiled at 140–147°, which was found to be isohexyl alcohol by converting it into 3,5-dinitrobenzoic ester. The boiling point mentioned above is lower and extends in wider range than the value in literature (b.p. 153°) and the sample is suspected to be mixed with isomeric secondary alcohol (inactive form, b.p. 129–134°) but the latter could not be identified. *Dinitrobenzoic ester.* It was prepared by treating the alcohol with 3,5-dinitrobenzoyl chloride according to the description of Mulliken.[10] The ester obtained melted at 68–69° after repeated recrystallisation from 95% alcohol (Table 2).

Fraction IV. After treating with 10% NaOH it was refractionated, b.p. 111–113.5° under 15 mm., 7.7 g. It was found to be isohexylene glycol by analysis (Table 3). *Bis-phenylurethane.* Mixture of the glycol (0.5 g.) and phenyl isocyanate (1.0 g.) was heated at 120–130° for 0.5 hour. On cooling, it changed to a viscous fluid. Ligroin (1:5) was added, well stirred, and colourless amorphous powder gradually deposited. It melted at 115–116° after recrystallisation from mixture of ligroin and alcohol (5:1) (Table 2).

Hydrogenation of Acetone-*dl*-mandelic acid. Experiment A. Acetone-compound (m.p. 46–47°) 38.5 g. (0.20 mol). Catalyzer 6.0 g. Time of hydrogenation 1.5 hours at 240–260°. Fractionation (filtered solution, 28.0 g.):

I	78– 80° under ordinary pressure	13.4 g.
II	30– 35° under 17 mm.	2.7 g.
III	102–107° under 14 mm.	8.4 g.

Fractions I and II had the odour of aromatic hydrocarbon. Twice its volume of water was added to them to dissolve isopropyl alcohol and the part not miscible with water was separated, which (5.8 g.) was distilled over metallic sodium. Even after repeated fractionation with the aid of Widmer column, it was not possible to separate it into fractions of distinct boiling points:

(a)	up to 110°	1.3 g.
(b)	110–114°	0.5 g.
(c)	115–122°	1.1 g.

An attempt was made to prepare crystalline nitro-compounds with the object of identifying the hydrocarbons, nitration being carried out, regarding (b) as toluene, by the method of Mulliken,[11] (c) as ethyl-benzene, according to the description of Schultz.[12] But the products thereby obtained always remained as oil and could not be brought into crystallisation. *Oxidation of* (a). It (0.8 g.) was boiled with potassium permanganate solution (3.0 g of salt in 75 c.c. of water) for one hour. After filtration and evaporation, the solution was acidified with hydrochloric acid. Crystals (0.1 g) which melted at 121° after recrystallisation from water were obtained and identified as benzoic acid by the mixed melting point determination. These fractions which gave benzoic acid on oxidation can be assumed to be a mixture of toluene and ethyl-benzene.

(10) Mulliken, "Identification of Organic Compounds," Vol. I, 168.
(11) Mulliken, *ibid.*, Vol. I, 202.
(12) G. Schultz, *Ber.*, **42** (1909), 2634.

Fraction II. Boiling point of this fraction corresponds to that of phenyl-ethyl alcohol and it was identified as such by converting it into phenylurethane. *Phenylurethane.* The alcohol (1.0 g.) and phenyl isocyanate (1.0 g.) were mixed and heated at 100–120° for one hour. On cooling, the mixture became a semi-fluid, to which an equal volume of petroleum ether was added, well stirred, and filtered. The crude urethane (1.5 g.) thus prepared was repeatedly recrystallized from alcohol. It melted at 78–79° (Table 2) and showed no lowering of melting point when mixed with a specimen of urethane prepared from pure phenyl-ethyl alcohol (Takeda).

Experiment B. Acetone-compound 25.2 g. (0.13 mol). Catalyzer 4.0 g. Time of hydrogenation 0.75 hour at 240–260°. Fractionation:

I	78– 80° under ordinary pressure	5.6 g.
II	105–106° under 16 mm.	5.4 g.
III	over 110° ,,	3.0 g.

In this experiment hydrogenation was interrupted before the absorption of hydrogen has been completed. The yield of fraction I (isopropyl alcohol and hydrocarbon) was inferior to that in Exp. A, while a fraction of higher boiling point (III) was obtained, which was not met with in Exp. A.

Fraction III has a odour reminding of aromatic ester. After removing acid by treating with 10% sodium carbonate, it was hydrolyzed by boiling with sodium hydroxide (10%) for three hours. The reaction mixture was acidified and extracted with ether. From the ether extract crystals (0.7 g.) were obtained, which melted at 75–76° after recrystallisation from water and were identified as phenyl-acetic acid by mixed melting point determination.

Hydrogenation of Acetone-*l*-phenyl-lactic acid. Experiment A. Acetone-compound (m.p. 63–64°) 25.0 g. (0.12 mol). Catalyzer 4.0 g. Time of hydrogenation 1.5 hours at 240–260°. Fractionation (15.1 g.):

I	77– 80° under ordinary pressure	2.5 g.
II	110–120° under 16 mm.	2.8 g.
III	120–123° ,,	2.4 g.
IV	164–166° ,,	6.3 g.

Fractions II and III and corresponding ones in the next experiment were combined and treated as follows. The substance has a pleasant odour reminding of cinnamic ester. It was boiled with 10% sodium hydroxide for one hour to hydrolyze the ester and was extracted with ether. The ether extract was separated into three fractions by fractionation:

(a)	up to 100°	under 9 mm.	2.4 g.
(b)	100–108°	,,	2.6 g.
(c)	108–109°	,,	6.2 g.

These fractions were found to be a mixture of phenyl-propyl alcohol (b.p. 120–121° under 13 mm.) and benzyl methyl carbinol (active form, b.p. 125° under 25 mm.) by converting them into phenylurethanes. *Phenylurethanes.* Though the differentiation of isomeric alcohols by above fractionation never seemed complete, their phenyl-

urethanes could be obtained separately from fractions rich in corresponding alcohols, as solubilities of the phenylurethanes in ethyl alcohol are markedly different from each other. The alcohol from fraction (a) (1.2 g.) and phenyl isocyanate (1.2 g.) were mixed and heated at 130° for two hours. On cooling, an equal volume of petroleum ether was added and the phenylurethane thus separated was filtered (1.2 g.). It melted at 88–89° after recrystallisations from alcohol (Table 2).

The alcohol from fraction (c) (1.0 g.) was treated with phenyl isocyanate just in the same way as above. On cooling, it was dissolved in alcohol, filtered and was concentrated. Phenylurethane gradually deposited after long standing. It melted at 45–46° after recrystallisations from little alcohol (Table 2) and showed no lowering when mixed with a specimen of urethane prepared from pure phenyl-propyl alcohol (Takeda, b.p. 110–113° under 9 mm.).

The alkaline solution used for hydrolysis of ester was acidified and extracted with ether. A small amount of crystals was obtained from the extract after long standing in the desiccator. It melted at 46–47° and was identified as hydrocinnamic acid by the mixed melting point determination.

Fraction IV. After treating with 10% sodium hydroxide it was extracted with ether. The extract was fractionated, and the main part distilled at 168–170° under 17 mm., which was found to be benzyl-ethylene glycol by analysis (Table 3) as well as by the formation of bis-phenylurethane (Table 2). *Bis-phenylurethane.* The glycol (1.0 g.) and phenyl isocyanate (2.0 g.) were mixed and heated at 120–130° for 0.5 hour. After cooling, twice its volume of ligroin was added, well stirred, from which crude urethane (2.6 g.) crystallized out on standing. It can be recrystallized from either alcohol or benzene. *Recrystallisation from alcohol.* Half amount of the crude urethane was dissolved in hot alcohol and filtered. The filtrate was concentrated to a syrup which gradually changed to crystalline mass. It melted at 108.5–109.5° after recrystallisation from the same solvent (A) (Table 2). *Recrystallisation from benzene.* Another half of the crude urethane was treated with benzene instead of alcohol just in the same way. The phenylurethane thus purified was amorphous powder and melted at 132–133° after recrystallisation from benzene (B) (Table 2).

Samples A and B showed the same nitrogen content corresponding to bis-phenylurethane, while their melting points were distinctly apart from each other. Sample A can be converted into B (melting at 129–131°) after one recrystallisation from benzene and the reverse change was observed when sample B was treated with alcohol (melting 109–110°). The monotropic transformation from A to B takes place on mere heating. Sample A was put in Abderhalden drying apparatus and maintained at 100° in vacuum for three hours and then at 110° for seven hours, during which the weight and melting point were determined from time to time. The loss of weight was never observed during whole operation, while melting point gradually changed and finally reached a constant value 131–133°, which shows that the sample was completely changed into B. The sample thus prepared can be reconverted into A (melting at 108–109°) by twice recrystallizing from alcohol.

Experiment B. Acetone-compound 25.0 g. (0.12 mol). Catalyzer 4.0 g. Time of hydrogenation 4.5 hours at 240–260°. Fractionation (19.3 g.):

I	79– 82° under ordinary pressure	7.5 g.
II	100–120° under 11 mm.	10.1 g.

Fraction I smelt of aromatic hydrocarbon as in the case of acetone-mandelic acid and when put into water, it did not dissolve completely, but the part insoluble in water was too minute to test its boiling point.

Fraction II was combined with the corresponding fraction in experiment A as already mentioned. In the present experiment higher boiling fraction corresponding to glycol was lacking. The glycol seemed to have been completely hydrogenated to monohydric alcohols in prolonged hydrogenation in this experiment.

Table 2.

Alcohol and glycol	Derivative prepared	N		
		Found*	Calc.	for
$CH_3 \cdot CH(OH) \cdot CH_3$	α-Naphthylurethane, m.p. 104–105°	6.07	$C_{14}H_{15}O_2N$,	6.11%
$(CH_3)_2CH \cdot CH_2 \cdot CH_2 \cdot CH_2OH$	3, 5-Dinitrobenzoic ester,[13] m.p. 68–69°	9.64	$C_{13}H_{16}O_6N_2$,	9.46%
$C_6H_5 \cdot CH_2 \cdot CH_2OH$	Phenylurethane, m.p. 78–79°	5.77	$C_{15}H_{15}O_2N$,	5.81%
$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2OH$	Phenylurethane, m.p. 44–45°	5.63	$C_{16}H_{17}O_2N$,	5.49%
$C_6H_5 \cdot CH_2 \cdot CH(OH) \cdot CH_3$ (active)**	Phenylurethane,[14] m.p. 88–89°	5.48	$C_{16}H_{17}O_2N$,	5.49%
$CH_3 \cdot CH(OH) \cdot CH_2OH$ (*dl*)	Bis-phenylurethane,[15] m.p. 143–144°	8.87	$C_{17}H_{18}O_4N_2$,	8.90%
$(CH_3)_2CH \cdot CH_2 \cdot CH(OH) \cdot CH_2OH$ (active)**	Bis-phenylurethane, m.p. 115–116°	7.79	$C_{20}H_{24}O_4N_2$,	7.87%
$C_6H_5 \cdot CH_2 \cdot CH(OH) \cdot CH_2OH$ (active)**	Bis-phenylurethane, {m.p. 108–109° (A) {m.p. 132–133° (B)	7.18 7.13	$C_{23}H_{22}O_4N_2$,	7.18%

* Nitrogen was estimated by micro-Kjeldahl method except in case of nitro-compound.

** The activities were not examined. During hydrogenation they might be subjected to racemisation to some extent. E. Bowden, H. Adkins, *J. Am. Chem. Soc.*, **56** (1934), 689.

(13) G. B. Malone, E. E. Reid, *J. Am. Chem. Soc.*, **51** (1929), 3426.

(14) Phenylurethane of active alcohol is unknown. That of *dl*-alcohol melts at 92°. M. Tiffeneau, *Compt. rend.*, **146** (1908), 698.

(15) G. S. Walpole, *Proc. Roy. Soc. London* (B), **83** (1911), 272. Walpole gives the m.p. 152.5–153.5° for the same bis-phenylurethane of *dl*-glycol, but in the present study it could not be raised above 144°. That of active glycol, m.p. 145–146°, P. A. Levene, A. Walti, *J. Biol. Chem.*, **73** (1927), 263.

Table 3.

Glycol	Analytical results	
	Found	Calc. for
Isohexylene glycol, b.p. 111–113.5° under 15 mm.	C, 60.82; H, 11.84	$C_6H_{14}O_2$: C, 60.95; H, 11.96%
Benzyl-ethylene glycol, b.p. 168–170° under 17 mm.	C, 70.94; H, 8.11	$C_9H_{12}O_2$: C, 71,01; H, 7.96%

The present study was carried out in the Chemical Institute, Faculty of Science, Imperial University of Tokyo. The author wishes to express his sincere thanks to Prof. K. Matsubara for the kind inspection of this paper. His best thanks are also due to Dr. Y. Takayama for the constant encouragements during this study.

THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL. IX. THE CONSTITUTION OF DOCOSAHEXENOIC ACID $C_{22}H_{32}O_2$.

By Yoshiyuki TOYAMA and Tomotaro TSUCHIYA.

Received July 31st, 1935. Published November 28th, 1935.

In previously reported studies[1] on the highly unsaturated C_{22}-acids in sardine oil, clupanodonic acid $C_{22}H_{34}O_2$ was isolated in much purer state than obtained before, and its constitution was established as $\Delta^{4:5,\ 8:9,\ 12:13,\ 15:16,\ 19:20}$-docosapentenoic acid. A concentrated fraction of docosahexenoic acid $C_{22}H_{32}O_2$ was also separated. The present paper records the experimental results obtained by the ozonolysis of the amyl ester prepared from the concentrated fraction of docosahexenoic acid.

Among the products of ozonolysis acetaldehyde $CH_3{\cdot}COH$, acetic acid $CH_3{\cdot}COOH$, carbon dioxide CO_2, succinic acid $HOOC{\cdot}(CH_2)_2{\cdot}COOH$ and amyl hydrogen succinate $HOOC{\cdot}(CH_2)_2{\cdot}COOC_5H_{11}$ were found; the presence of the aldehydes corresponding to succinic acid and amyl hydrogen succinate was also indicated. Although the amyl ester used for ozonolysis was contaminated with a small proportion of

(1) This Bulletin, **10** (1935), 433, 441.

amyl clupanodonate which yielded on ozonolysis the above compounds as shown in a preceding paper,[2] the yields of the above compounds obtained in these experiments indicated that they owed their origin largely to amyl docosahexenoate which constituted the bulk of the amyl ester used for ozonolysis. The volatile aldehydes formed by the ozonolysis appeared not to consist exclusively of acetaldehyde, but no other volatile aldehydes than acetaldehyde were identified.[3] Of the products of ozonolysis obtained in these experiments, succinic acid and amyl hydrogen succinate are derived from the groups $=CH\cdot(CH_2)_2\cdot CH=$ and $=CH\cdot(CH_2)_2\cdot COOC_5H_{11}$ respectively, whilst carbon dioxide is formed, together with acetaldehyde and acetic acid, by the secondary decomposition of the products of ozonolysis derived from the group $=CH\cdot CH_2\cdot CH=$, and the yield of carbon dioxide indicated the presence of at least three of the group $=CH\cdot CH_2\cdot CH=$ in amyl docosahexenoate. Accordingly the free docosahexenoic acid was found to contain the following groups: $=CH\cdot(CH_2)_2\cdot CH=$, $=CH\cdot CH_2\cdot CH=$ (at least three), and $=CH\cdot(CH_2)_2\cdot COOH$. Although the group attached to the opposite side of the carboxyl group was left undetermined in these experiments, it must be $CH_2=$ or $CH_3\cdot CH=$ depending on whether docosahexenoic acid has three or four of the group $=CH\cdot CH_2\cdot CH=$, since it has 22 carbon atoms in total. Hence, docosahexenoic acid is composed of either one of the following two sets of the groups:

(1) $CH_2=$, $=CH\cdot CH_2\cdot CH=$(three), $=CH\cdot(CH_2)_2\cdot CH=$(two), $=CH\cdot(CH_2)_2\cdot COOH$.
(2) $CH_3\cdot CH=$, $=CH\cdot CH_2\cdot CH=$(four), $=CH\cdot(CH_2)_2\cdot CH=$, $=CH\cdot(CH_2)_2\cdot COOH$.

No evidence was brought out in these experiments regarding the respective positions of the groups $=CH\cdot CH_2\cdot CH=$ and $=CH\cdot(CH_2)_2\cdot CH=$. If, however, the group $=CH\cdot(CH_2)_2\cdot CH=$ is assumed to lie near the carboxyl group side of docosahexenoic acid, as is the case with previously studied moroctic,[4] eicosatetraenoic[5] and clupanodonic acids,[6] the constitution of docosahexenoic acid is to be expressed by either one of the following formulae:

$$CH_2=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot(CH_2)_2\cdot CH=$$
$$CH\cdot(CH_2)_2\cdot CH=CH\cdot(CH_2)_2\cdot COOH \quad (I)$$

$$CH_3\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=$$
$$CH\cdot(CH_2)_2\cdot CH=CH\cdot(CH_2)_2\cdot COOH \quad (II)$$

(2) This Bulletin, **10** (1935), 441.

(3) Propyl aldehyde must have been formed, though in a small amount, by the ozonolysis of the amyl ester, since the ester was contaminated with amyl clupanodonate.

(4) This Bulletin, **10** (1935), 232.

(5) This Bulletin, **10** (1935), 296.

(6) This Bulletin, **10** (1935), 441.

The yield of C_4-compounds obtained by the ozonolysis could not be determined accurately, but it seemed to indicate the presence of more than one of the group $=CH\cdot(CH_2)_2\cdot CH=$ in amyl docosahexenoate. Accordingly the formula (I) seems most probable. Further experiments will be done when pure docosahexenoic acid is obtained in sufficient quantity.

Experimental.

Docosahexenoic acid used in these experiments is the same sample which was obtained in the previous study.[7] It had d_4^{15} 0.9521, n_D^{15} 1.5129, neutr. value 170.1 (calc. 170.9), iodine value by the Wijs method 456.0 (calc. 464.0) and gave 177% of an ether insoluble bromide having Br-content 74.00% (calc. for $C_{22}H_{32}O_2Br_{12}$: 74.50%). Assuming that this sample is contaminated exclusively with clupanodonic acid, the purity of docosahexenoic acid is calculated to be 89.97% from its iodine value. The free acid was converted into its amyl ester by heating with an equal amount of amyl alcohol containing 2.5% of hydrogen chloride on the water-bath under a reflux condenser for one hour. Five g. of the amyl ester thus obtained was dissolved in 50 c.c. of chloroform, cooled with ice-salt, and a current of ozonised oxygen was passed into the solution until it became saturated with ozone. On distilling chloroform from the solution under diminished pressure, there was obtained 8.9 g. of ozonide as an orange yellow syrup. Chloroform was, however, not thoroughly removed from the ozonide, since there was a danger of explosive decomposition on undue heating. Water (50 c.c.) was added to the ozonide, and the liquid was heated on the water-bath in a current of hydrogen. The flask was attached by a delivery tube to other three flasks (**a**, **b**, and **c**) which were connected in succession; the first (**a**) was filled with ice cold water, the second (**b**) and the third (**c**) with approximately 1/3 N barium hydroxide solution. The volatile decomposition products (**A**) carried over with hydrogen were passed into the above three flasks where they were caught by absorption. The decomposition products remained in the initial flask dissolved partly in water, and the remainder separated as oily substances (**C**) under aqueous solution (**B**).

1. **Volatile Decomposition Products (A).** The aqueous solution in the flask (**a**) produced a pink colouration with Schiff's reagent and a deep blue colouration with diethylamine and sodium nitroprusside, indicating the presence of acetaldehyde. On addition of *p*-nitrophenylhydrazine and hydrochloric acid, there was formed a precipitate of *p*-nitrophenylhydrazone which on recrystallisation from 50% alcohol gave yellow needles with m.p. 125–126° which was not lowered when the substance was admixed with a pure specimen of *p*-nitrophenylhydrazone of acetaldehyde (m.p. 128°) in various proportions.

The barium hydroxide solution in the flasks (**b**) and (**c**) were found to contain a precipitate of barium carbonate, indicating that carbon dioxide was formed by the ozonolysis. The quantity of carbon dioxide calculated from the quantity of barium carbonate was found to be 1.26 g. or 25.2% of the amyl ester used for ozonolysis.

(7) This Bulletin, **10** (1935), 433.

This is higher than the maximum yield (22.1%) of carbon dioxide obtainable on assumption that amyl docosahexenoate has two of the group $=CH\cdot CH_2\cdot CH=$, and that the products of ozonolysis derived from that group undergoes secondary decomposition with the formation of carbon dioxide to a quantitative extent. Consequently amyl docosahexenoate must have at least three of the group $=CH\cdot CH_2\cdot CH=$.

2. **Aqueous Solution (B).** This was extracted with a large quantity of ether. On removal of ether from the ethereal extract, there remained 3.6 g. of a reddish orange liquid, which was then treated with petroleum ether and was separated into petroleum ether solution and petroleum ether insoluble portion. On distilling off petroleum ether from the solution, the distillate (petroleum ether) had a smell of acetic acid. The residue was then heated in an oil-bath, yielding 0.6 g. of a colourless distillate below 150° of the bath-temperature. It had a smell of acetic acid, and the silver salt prepared from it was proved to be silver acetate by analysis (Found: Ag, 64.48. Calc. for $C_2H_3O_2Ag$, 64.64%).

The petroleum ether insoluble portion (1.9 g.) consisted of a mixture of reddish orange liquid and crystalline solid, and showed aldehydic character. Oxidation with an alkaline solution of potassium permanganate followed by acidification yielded a crystalline product which, after being washed with a little cold ether, showed neutr. value 947.6 (calc. for succinic acid $C_4H_6O_4$: 950.6) and m.p. 179–180°. Recrystallisation from ethyl acetate yielded pure succinic acid with m.p. and mixed m.p. 182–183°. The petroleum ether insoluble portion was considered to consist almost exclusively of C_4-compounds (succinic acid and the corresponding aldehyde), and the yield was 1.9 g. or 38% of the amyl ester used for ozonolysis. This is considerably higher than the maximum yield of C_4-compounds (as succinic acid) 27.1% which is obtainable on condition that amyl docosahexenoate has only one of the group $=CH\cdot(CH_2)_2\cdot CH=$.

3. **Oily Substances (C).** Yield 1.9 g. These substances had acid and aldehydic characters. Oxidation with alkaline permanganate followed by acidification yielded an acid ester which showed neutr. value 297.1 and saponification value 594.8, and was considered to be amyl hydrogen succinate $C_9H_{16}O_4$ (neutr. value 298.2 and saponif. value 596.5). The free acid liberated from its acid ester in the usual way yielded succinic acid after recrystallisation from ethyl acetate; neutr. value 946.9 and m.p. 182–183°. From these results it is seen that the oily substances consist chiefly of amyl hydrogen succinate and the corresponding aldehyde.

Summary.

1. A concentrated fraction of docosahexenoic acid was converted into the amyl ester, and the latter was subjected to ozonolysis. Among the products of ozonolysis were found: acetaldehyde, acetic acid, carbon dioxide, succinic acid and amyl hydrogen succinate. The presence of the aldehydes corresponding to succinic acid and amyl hydrogen succinate was also indicated. Of these compounds, carbon dioxide together with acetaldehyde and acetic acid is attributable to the secondary decomposition of the products derived from the group $=CH\cdot CH_2\cdot CH=$. The yield of carbon dioxide indicated the presence of at least three of the group

$=CH\cdot CH_2\cdot CH=$ in amyl docosahexenoate. Consequently docosahexenoic acid has the following groups: $=CH\cdot CH_2\cdot CH=$, $=CH\cdot(CH_2)_2\cdot CH=$, and $=CH\cdot(CH_2)_2\cdot COOH$, of which it has at least three of the group $=CH\cdot CH_2\cdot CH=$. The yield of C_4-compounds (succinic acid and the corresponding aldehyde) obtained by the ozonolysis gave an indication of the presence of more than one of the group $=CH\cdot(CH_2)_2\cdot CH=$ in docosahexenoic acid.

2. Although the group attached to the CH_3-side and the respective positions of the groups $=CH\cdot CH_2\cdot CH=$ and $=CH\cdot(CH_2)_2\cdot CH=$ were left undetermined in these experiments, the most probable structure of docosahexenoic acid was derived from the results obtained above.

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Correction to the 7th paper on the Highly Unsaturated Acids in Sardine Oil:

Read n_D^{20} 1.5014 *for* n_D^{21} 1.4934 *in line 16 from bottom on page 439 of this volume.*

THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL X. THE SEPARATION OF HIGHLY UNSATURATED C_{24}-ACIDS.

By **Yoshiyuki TOYAMA** and **Tomotaro TSUCHIYA.**

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After having examined the highly unsaturated C_{16}-, C_{18}-, C_{20} and C_{22}- acids in sardine oil, we extended our investigations to the highly unsaturated C_{24}-acids. Concerning the highly unsaturated C_{24}-acids in sardine oil, nothing has hitherto been known. However, the highly unsaturated C_{24}-acids in marine animal oils other than sardine oil have been studied by a few authors. Bull[1] stated to have found an acid $C_{24}H_{40}O_2$ in herring oil. Ueno and Iwai[2] have recently shown the presence of a highly unsaturated acid $C_{24}H_{38}O_2$, which they named scoliodonic acid, in

(1) *Chem. Ztg.*, **23** (1899), 996.
(2) *J. Soc. Chem. Ind., Japan*, **37** (1934), 562.

the liver oil of *Scoliodon laticaudus*. We[3] separated a highly unsaturated acid $C_{24}H_{36}O_2$, named nisinic acid, from herring oil, and found the same acid also in cod-liver oil, pilot-whale oil and in the liver oil from *Squalus sucklii*.

For the separation of highly unsaturated C_{24}-acids in the present experiment, the residue from the distillation of the highly unsaturated methyl esters described in the 7th report[4] of this series was used as the starting material. The fatty acids liberated from it consisted mainly of highly unsaturated C_{24}-acids and some polymerised acids. These were separated into the fractions of different degrees of unsaturation by the fractional precipitation of sodium soaps in acetone, and there was obtained nisinic acid $C_{24}H_{36}O_2$[5] from the fraction of the highest degree of unsaturation. Examination of each fraction seemed to indicate the presence of some other highly unsaturated C_{24}-acids, such as scoliodonic acid $C_{24}H_{38}O_2$ and also an acid $C_{24}H_{40}O_2$, besides nisinic acid, but no individual acid other than nisinic acid was separated.

Experimental.

1. **Separation of Highly Unsaturated C_{24}-Acids.** In the previously reported experiment[6] of the separation of highly unsaturated C_{22}-acids, 10 kg. of sardine oil was treated by means of sodium-soap-acetone method, and the concentrated fraction of highly unsaturated acids was obtained from the sodium soaps soluble in acetone. This was converted into the methyl esters and the latter were subjected to the fractional distillation which was continued up to 214°/2 mm. For the present experiment the residue from the distillation was used as the starting material. It was saponified, the unsaponifiable matter was removed by extraction with ether, and the soap solution on acidification yielded free fatty acids. These were reconverted into the methyl esters and the latter were distilled up to 215°/2 mm., yielding about 69 g. of a residue which had saponif. value 150.5 and iodine value 308.3.[7] The residue was saponified, and the fatty acids obtained on acidification were treated with charcoal in petroleum ether. The fatty acids (62. g.) thus obtained showed neutr. value 156.8 and iodine value 324.5. The product of hydrogenation of these fatty acids showed neutr. value 151.5 (calc. for $C_{24}H_{48}O_2$: 152.3) and m.p. 81.5–82° after recrystallisation from alcohol. The melting point was not lowered when the substance was admixed in various proportions with a specimen of *n*-tetracosanic acid, m.p. 84–84.5°, prepared from behenic acid

(3) *J. Soc. Chem. Ind., Japan*, **37** (1934), 1176.

(4) This Bulletin, **10** (1935), 433.

(5) Strictly speaking, nisinic acid or an acid having the same composition as nisinic acid.

(6) This Bulletin, **10** (1935), 433.

(7) Unless otherwise stated, the iodine values recorded in this paper were determined by the Wijs method.

by malonic ester synthesis. It follows from these results that the fatty acids having m.p. 81.5–82° consist mainly of *n*-tetracosanic acid, and consequently the highly unsaturated acids prior to hydrogenation consist chiefly of C_{24}-acids.

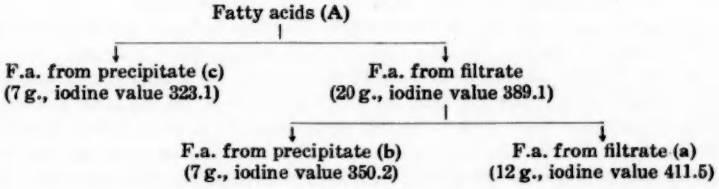
2. **Fractional Precipitation of Sodium Soaps in Acetone.** The highly unsaturated C_{24}-acids (neutr. value 156.8 and iodine value 324.5) obtained above were neutralised with a portion of sodium hydroxide solution prepared by dissolving 10 g. of sodium hydroxide in 20 c.c. of water and 40 c.c. of 95% alcohol, and then 60 c.c. of acetone was added by which a precipitate of insoluble sodium soaps was formed. The precipitate was filtered, and the fatty acids were separated from the precipitate and the filtrate, respectively, in the usual way. Fifty-nine grams of the highly unsaturated C_{24}-acids gave the following results (Table 1).

Table 1.

	Yield (g.)	Iodine value
Fatty acids from filtrate (A)	29	371.7
Fatty acids from precipitate (B)	29	277.6
Loss	1	—

Fatty acids from filtrate (A). These were dissolved in a little acetone, partially neutralised by using a portion of sodium hydroxide solution prepared by dissolving 10 g. of sodium hydroxide in 10 c.c. of water and 20 c.c. of 95% alcohol, and a sufficient quantity of acetone was added, by which a precipitate of insoluble sodium soaps was formed. The precipitate was once brought into solution by warming the solution on the water-bath, reprecipitated on cooling, and then filtered. The fatty acids were separated from the precipitate and the filtrate, respectively, in the usual way, and those obtained from the filtrate were again treated as before to effect a further separation. The results of these treatments are given below (Table 2).

Table 2.


Fatty acids (A)
F.a. from precipitate (c)
(7 g., iodine value 323.1)
F.a. from filtrate
(20 g., iodine value 389.1)
F.a. from precipitate (b)
(7 g., iodine value 350.2)
F.a. from filtrate (a)
(12 g., iodine value 411.5)

For the sake of comparison, the neutralisation value and the iodine value for the highly unsaturated C_{24}-acids and also the Br-content of the corresponding bromo-derivatives are shown in Table 3.

The iodine value of the fatty acids (a) is close to the calculated value for $C_{24}H_{36}O_2$. The fatty acids (b) showed an iodine value which was close to the calculated value for $C_{24}H_{38}O_2$, but the ether insoluble bromide obtained from them showed

Table 3.

Acid	Neutr. v.	Iodine v.	Bromo-derivative	Br-content (%)
$C_{24}H_{36}O_2$	157.5	427.5	$C_{24}H_{36}O_2Br_{12}$	72.91
$C_{24}H_{38}O_2$	156.6	354.2	$C_{24}H_{38}O_2Br_{10}$	69.05
$C_{24}H_{40}O_2$	155.7	281.8	$C_{24}H_{40}O_2Br_8$	63.96
$C_{24}H_{42}O_2$	154.9	210.2	$C_{24}H_{42}O_2Br_6$	56.96

Br-content 70.84%, which lies between the calculated values for $C_{24}H_{36}O_2Br_{12}$ and $C_{24}H_{38}O_2Br_{10}$. Accordingly the fatty acids (b) are believed to be a mixture of acids of different degrees of unsaturation. The fatty acids (c) seem to contain some less unsaturated acids than $C_{24}H_{38}O_2$ according to their iodine value.

The fatty acids (a) were partially neutralised with sodium hydroxide solution in acetone as before, and the precipitate of insoluble sodium soaps was removed. The fatty acids obtained from the filtrate were again subjected to the same treatment, and these separative operations were repeated several times, until the fatty acids obtained from the precipitate and the filtrate showed iodine values which did not differ much from one another. The fatty acids from the final filtrate had the following constants and were considered to consist chiefly of nisinic acid $C_{24}H_{36}O_2$: d_4^{15} 0.9486, d_4^{20} 0.9451, n_D^{15} 1.5140, n_D^{20} 1.5120, mol. refraction 113.1 (calc. for $C_{24}H_{36}O_2$: 111.8), neutr. value 157.0, iodine values by the Wijs and the Rosenmund-Kuhnhenn methods 422.5 and 407.2 respectively. Bromination in ethereal solution yielded 138% of an ether insoluble bromide which showed Br-content 72.76% and turned black at about 240° without melting.

Fatty acids from precipitate (B). Twenty-eight grams of these fatty acids were neutralised by using a portion of sodium hydroxide solution prepared by dissolving 10 g. of sodium hydroxide in 30 c.c. of water and 60 c.c. of 95% alcohol, and then 300 c.c. of acetone was added by which a precipitate of insoluble sodium soaps was formed. The fatty acids (18.5 g.) liberated from the insoluble sodium soaps had iodine value 260.1 and n_D^{15} 1.5208. The iodine value lies between the calculated values for $C_{24}H_{40}O_2$ and $C_{24}H_{42}O_2$. Bromination in ethereal solution yielded, however, an ether insoluble bromide having Br-content 68.09% which was higher than the calculated value for $C_{24}H_{40}O_2Br_8$, indicating that the fatty acids from the insoluble sodium soaps contained some more highly unsaturated acids than $C_{24}H_{40}O_2$. After distilling off the solvent from the filtrate from ether insoluble bromide, the residue was treated with petroleum ether, and there was separated a petroleum ether insoluble bromide which melted at about 70° and had Br-content 57.55% which was close to the calculated value for $C_{24}H_{42}O_2Br_6$. It should, however, be noted that the fatty acids obtained from the insoluble sodium soaps showed an abnormally high refractive index in comparison with their iodine value, giving an indication of contamination of some polymerised fatty acids, and consequently it is not excluded that the petroleum ether insoluble bromide obtained above may have been derived from polymerised fatty acids, but not from $C_{24}H_{42}O_2$.

Summary.

A concentrated fraction of highly unsaturated acids was separated from sardine oil by means of sodium-soap-acetone method, and was distilled as its methyl esters up to 215°/2 mm. The residue from the distillation consisted mainly of the methyl esters of highly unsaturated C_{24}-acids and some polymerised products. The free fatty acids liberated from the residue were separated into the fractions of different degrees of unsaturation by means of fractional precipitation of sodium soaps in acetone. The fraction of the highest degree of unsaturation consisted of nisinic acid $C_{24}H_{36}O_2$. Examination of other fractions seemed to indicate the presence of some less unsaturated acids, such as $C_{24}H_{38}O_2$ and $C_{24}H_{40}O_2$, but no individual acids other than nisinic acid were separated.

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THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL. XI. THE CONSTITUTION OF NISINIC ACID $C_{24}H_{36}O_2$ IN SARDINE OIL.

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As stated in the 10th report of this series,[1] nisinic acid $C_{24}H_{36}O_2$ was separated as an individual component of highly unsaturated C_{24}-acids in sardine oil. In continuation to the previous study this paper deals with the experimental results obtained by the ozonolysis of amyl nisinate with a view to gain an insight into the constitution of nisinic acid.

On examining the products obtained by the ozonolysis of amyl nisinate, the following compounds were found: propyl aldehyde $CH_3{\cdot}CH_2{\cdot}COH$, propionic acid $CH_3{\cdot}CH_2{\cdot}COOH$, acetaldehyde $CH_3{\cdot}COH$, acetic acid $CH_3{\cdot}COOH$, carbon dioxide CO_2, succinic acid $HOOC{\cdot}(CH_2)_2{\cdot}COOH$ and amyl hydrogen succinate $HOOC{\cdot}(CH_2)_2{\cdot}COOC_5H_{11}$.

(1) This Bulletin, **10** (1935), 543.

Of these compounds, propyl aldehyde and propionic acid are derived from the group $CH_3{\cdot}CH_2{\cdot}CH=$. Acetaldehyde and acetic acid together with carbon dioxide are attributable to the secondary decomposition of malonic acid and the corresponding aldehyde which are derived from the group $=CH{\cdot}CH_2{\cdot}CH=$, while succinic acid and amyl hydrogen succinate are derived from the groups $=CH{\cdot}(CH_2)_2{\cdot}CH=$ and $=CH{\cdot}(CH_2)_2{\cdot}COOC_5H_{11}$ respectively. Accordingly, amyl nisinate was found to contain the following groups: $CH_3{\cdot}CH_2{\cdot}CH=$, $=CH{\cdot}CH_2{\cdot}CH=$, $=CH{\cdot}(CH_2)_2{\cdot}CH=$ and $=CH{\cdot}(CH_2)_2{\cdot}COOC_5H_{11}$. It was also found from the yield of carbon dioxide obtained by the ozonolysis that amyl nisinate contained more than two of the group $=CH{\cdot}CH_2{\cdot}CH=$. These results, coupled with the fact that nisinic acid consists of 24 carbon atoms, show that nisinic acid is composed of the following groups: $CH_3{\cdot}CH_2{\cdot}CH=$, three of $=CH{\cdot}CH_2{\cdot}CH=$, two of $=CH{\cdot}(CH_2)_2{\cdot}CH=$, and $=CH{\cdot}(CH_2)_2{\cdot}COOH$. No evidence was brought out in these experiments regarding the respective positions of the groups $=CH{\cdot}CH_2{\cdot}CH=$ and $=CH{\cdot}(CH_2)_2{\cdot}CH=$. If, however, it is assumed that nisinic acid has ethylenic linkings in 4:5-, 8:9- and 12:13-positions as is the case with the previously studied moroctic acid ($\Delta^{4:5,\ 8:9,\ 12:13,\ 15:16}$),[2] eicosatetraenoic acid ($\Delta^{4:5,\ 8:9,\ 12:13,\ 16:17}$),[3] and clupanodonic acid ($\Delta^{4:5,\ 8:9,\ 12:13,\ 15:16,\ 19:20}$),[4] the constitution of nisinic acid should be represented by the following formula:

$$CH_3{\cdot}CH_2{\cdot}CH=CH{\cdot}CH_2{\cdot}CH=CH{\cdot}CH_2{\cdot}CH=CH{\cdot}CH_2{\cdot}CH=CH{\cdot}(CH_2)_2{\cdot}CH$$
$$=CH{\cdot}(CH_2)_2{\cdot}CH=CH{\cdot}(CH_2)_2{\cdot}COOH.$$

Experimental.

1. Separation of Nisinic Acid. In previously reported experiments of the separation of hiragonic, moroctic and eicosatetraenoic acids, 60 kg. of the ethyl ester prepared from sardine oil had been distilled, yielding two fractions boiling below and above 215°/10 mm. respectively. The distillation had been continued up to 225°/3 mm., leaving ca. 4 kg. of residue. For the present experiments, 1.5 kg. of this residue was used as the starting material. It was first treated by means of the sodium-soap-acetone method; the sodium soaps insoluble in acetone were removed, and the highly unsaturated acids were obtained from the sodium soaps soluble in acetone in the usual way. These were converted into the methyl esters, and the latter were subjected to distillation by which all the esters boiling below 215°/2 mm. were removed. The residue was saponified, the unsaponifiable matter was removed by extraction with ether, and the fatty acids liberated from the soap solution on acidification were treated with decolorizing carbon in petroleum ether. After these treatments there were obtained 145 g. of fatty acids having neutralisation value 155.8 and

(2) This Bulletin, **10** (1935), 232.
(3) This Bulletin, **10** (1935), 296.
(4) This Bulletin, **10** (1935), 441.

iodine value 319.1.[5] In order to effect a further separation of these fatty acids into individual components, they were dissolved in acetone and partially neutralised with sodium hydroxide solution in a similar manner as described in the previous experiment.[6] The precipitate of insoluble sodium soaps was filtered, and the fatty acids were recovered from the filtrate. These were again subjected to a fractional precipitation of sodium soaps in acetone, and the less highly unsaturated portion was removed as sodium soaps insoluble in acetone. After repeating these separative operations several times there were obtained finally 25 g. of fatty acids having iodine value 397.8 from the filtrate. These were brominated in ethereal solution, and the ether insoluble bromide was separated. On debrominating the ether insoluble bromide with zinc powder and glacial acetic acid, there were obtained 6.6 g. of fatty acids which had neutr. value 155.4 and iodine value 419.1, and were considered to consist mainly of nisinic acid. They were united with the sample (7 g.) of nisinic acid (iodine value 422.5) obtained in the previous experiment[7] and converted into the methyl esters, and the latter were subjected to distillation by which a fraction boiling at 198–204°/0.4 mm. was taken as methyl nisinate $C_{25}H_{38}O_2$. It had the following constants: d_4^{15} 0.9342, d_4^{20} 0.9307, n_D^{15} 1.5064, n_D^{20} 1.5044, saponif. value 151.1 (cal. 151.5), iodine value 408.5 (calc. 411.3).

Nisinic acid $C_{24}H_{36}O_2$ liberated from its methyl ester had the following constants: d_4^{15} 0.9487, d_4^{20} 0.9452, n_D^{15} 1.5143, n_D^{20} 1.5122, neutr. value 157.0 (calc. 157.5), iodine values by the Wijs and the Rosenmund-Kuhnhenn methods 424.5 and 410.3 (calc. 427.5) respectively.

On brominating nisinic acid in ethereal solution it yielded 140% of an ether insoluble bromide which had Br-content 72.78% (calc. for $C_{24}H_{36}O_2Br_{12}$: 72.91%) and turned black at about 240° without melting. The hydrogenation product of nisinic acid crystallised from alcohol in lustrous laminae which showed neutr. value 152.4 (calc. for $C_{24}H_{48}O_2$: 152.3) and m.p. 82.5–83°. The melting point was not lowered when the substance was admixed with various proportions of a specimen of *n*-tetracosanic acid $C_{24}H_{48}O_2$ which was prepared from behenic acid by malonic ester synthesis and melted at 84–84.5°.

2. **Ozonolysis of Amyl Nisinate.** Nisinic acid obtained above was heated with an equal amount of amyl alcohol containing 2.5% of hydrogen chloride on the water-bath under a reflux condenser for one hour. The resulting solution containing amyl nisinate was taken up with ether, and the ethereal solution was washed with sodium carbonate solution in order to remove hydrogen chloride and the unchanged nisinic acid, and then washed thoroughly with water. On distilling off ether and amyl alcohol from the ethereal solution, there remained amyl nisinate. Nine grams of the amyl nisinate was dissolved in 90 c.c. of chloroform, and a current of ozonised oxygen was passed into the solution under cooling with ice-salt until the solution became saturated with ozone. On removal of chloroform under diminished pressure there was obtained 16.2 g. (or 180%) of ozonide as an orange yellow syrup. The ozonide was, however, not thoroughly freed from chloroform, since there was a danger of frothing towards the end of the distillation of chloroform. The theoretical yields of ozonides obtainable from amyl nisinate are 167.5% for normal ozonide $C_{29}H_{46}O_{20}$ and 171.3% for ozonide

(5) Unless otherwise stated, the iodine values were determined by the Wijs method.

(6), (7) This Bulletin, **10** (1935), 543.

peroxide $C_{20}H_{40}O_{20}$. Water (90 c.c.) was added to the ozonide, and the liquid was heated on the water-bath for 30 minutes. The flask containing the ozonide was attached to other two flasks (a and b) in succession by means of a delivery tube; the first (a) was filled with ice-cold water, and the second (b) with barium hydroxide solution. During heating a current of hydrogen was passed through the flask containing the ozonide, and the volatile products (A) carried over with hydrogen were caught by absorption in the above two flasks. The products of ozonolysis remaining in the initial flask dissolved partly in water, and the remainder separated as oily substances (C) under aqueous solution (B). Each portion (A, B and C) was examined in the following manner.

(i) **Volatile products (A).** The aqueous solution in the flask (a) produced a pink colouration with Schiff's reagent, and a deep blue colouration with sodium nitroprusside and diethylamine, indicating the presence of acetaldehyde. When the phenylhydrazone prepared from this solution was heated with zinc chloride at 180°, a smell of scatol was recognised, which indicated the presence of propyl aldehyde. A partial separation of two aldehydes was attempted by warming a portion of the solution in a flask (a_1) at 45°, and passing a current of carbon dioxide into the flask so as to drive off the volatile substances into other flask (a_2) which was connected with the initial flask by means of a delivery tube. The flask (a_2) was filled with ice-cold water, which served to absorb the volatile substances carried over with carbon dioxide. The *p*-nitrophenylhydrazone prepared from the solution in the flask (a_1) melted at 120–121° after recrystallisation from 50% alcohol, and was considered to consist largely of *p*-nitrophenylhydrazone of propyl aldehyde (Found: N, 21.84. Calc. for $C_9H_{11}O_2N_3$: N, 21.76%). The solution in the flask (a_2) yielded *p*-nitrophenylhydrazone, which melted at 115–116° after recrystallisation from 50% alcohol and seemed to be a mixture of *p*-nitrophenylhydrazones of two aldehydes (Found: N, 22.95. Calc. for $C_9H_9O_2N_3$: N, 23.46. Calc. for $C_9H_{11}O_2N_3$: N, 21.76%).

The aqueous solution in the flask (a) showed also an acid reaction, and the presence of acetic acid was inferred from a red colouration developed on addition of ferric chloride to the neutralised solution.

The barium hydroxide solution in the flask (b) was found to contain a precipitate of barium carbonate, indicating the formation of carbon dioxide by the ozonolysis. Calculating from the quantity of the precipitate of barium carbonate, the yield of carbon dioxide formed by the ozonolysis was found to be 23.5% of amyl nisinate used for the ozonolysis. This is higher than the maximum yield 20.64% of carbon dioxide obtainable on assumption that amyl nisinate contains two of the group $=CH\cdot CH_2\cdot CH=$, and consequently amyl nisinate must have more than two of the group $=CH\cdot CH_2\cdot CH=$.

(ii) *Aqueous solution* (B). The products of ozonolysis dissolved in the aqueous solution (B) were extracted by shaking the solution with 900 c.c. of ether, and on removal of ether from the ethereal solution there remained 4.1 g. of an orange yellow liquid. This was washed three times with petroleum ether using 50 c.c. each time, and the petroleum ether solution and the insoluble portion were separated. The petroleum ether solution was heated on the water-bath to drive off petroleum ether, and the residue was then heated in the oil-bath yielding 0.7 g. of colourless distillate before the temperature of the bath reached 180°, when the distillation was discontinued. The distillate showed an acid reaction. The silver salts prepared from it were fractionally precipitated, yielding two crops which were found to be mixtures of silver

acetate and silver propionate by analyses (Found for 1st crop: Ag, 61.64. Found for 2nd crop: Ag, 63.59. Calc. for $C_2H_3O_2Ag$: Ag, 64.64. Calc. for $C_3H_5O_2Ag$: Ag, 59.63%).

The portion insoluble in petroleum ether (2.5 g.) was an orange yellow liquid. Oxidation with an alkaline solution of potassium permanganate followed by acidification gave a product consisting mostly of crystalline solid. It crystallised from ethyl acetate in needles having neutr. value 947.5 (calc. for succinic acid $C_4H_6O_4$: 950.6) and m.p. 181–182°. The melting point was not lowered when the substance was admixed with a pure specimen of succinic acid (m.p. 182–183°).

(iii) *Oily substances* (C). Yield 3.1 g. These were oxidised with alkaline permanganate. The product obtained after acidification had neutr. value 303.1 and saponif. value 590.5, and seemed to consist mainly of amyl hydrogen succinate $C_9H_{16}O_4$ (neutr. value 298.3, saponif. value 596.5). The free acid liberated from the acid ester yielded succinic acid after recrystallisation from ethyl acetate; neutr. value 948.1 and m.p. 181–182°.

Summary.

Nisinic acid $C_{24}H_{36}O_2$ has been separated from sardine oil, and its amyl ester subjected to ozonolysis. Among the products of ozonolysis were found: propyl aldehyde, propionic acid, acetaldehyde, acetic acid, carbon dioxide, succinic acid and amyl hydrogen succinate. Of these compounds propyl aldehyde and propionic acid are derived from the group $CH_3{\cdot}CH_2{\cdot}CH=$, whilst acetaldehyde and acetic acid together with carbon dioxide are attributable to the secondary decomposition of malonic acid and the corresponding aldehyde which are derived from the group $=CH{\cdot}CH_2{\cdot}CH=$. The yield of carbon dioxide indicated the presence of more than two of the group $=CH{\cdot}CH_2{\cdot}CH=$. Succinic acid and amyl hydrogen succinate are derived from the groups $=CH{\cdot}(CH_2)_2{\cdot}CH=$ and $=CH{\cdot}(CH_2)_2{\cdot}COOC_5H_{11}$ respectively. Accordingly, nisinic acid was found to be composed of the following groups: $CH_3{\cdot}CH_2{\cdot}CH=$, $=CH{\cdot}CH_2{\cdot}CH=$ (three), $=CH{\cdot}(CH_2)_2{\cdot}CH=$ (two), and $=CH{\cdot}(CH_2)_2{\cdot}COOH$. The respective positions of the groups $=CH{\cdot}CH_2{\cdot}CH=$ and $=CH{\cdot}(CH_2)_2{\cdot}CH=$ were not determined in these experiments. If, however, it is assumed that three of the ethylenic linkings in nisinic acid lie at 4:5-, 8:9- and 12:13-positions as is the case with previously studied moroctic, eicosatetraenoic and clupanodonic acids, the constitution of nisinic acid is expressed by the following formula:

$$CH_3{\cdot}CH_2{\cdot}CH=CH{\cdot}CH_2{\cdot}CH=CH{\cdot}CH_2{\cdot}CH=CH{\cdot}CH_2{\cdot}CH=CH{\cdot}(CH_2)_2{\cdot}CH=$$
$$CH{\cdot}(CH_2)_2{\cdot}CH=CH{\cdot}(CH_2)_2{\cdot}COOH.$$

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ISOTOPENAUSTAUSCH IN SOJABOHNEN.

Von Kenzo OKABE und Toshizo TITANI.

Eingegangen am 18. September 1935. Ausgegeben am 28. November 1935.

Auf die Austauschreaktionen zwischen H- und D-Atomen im Organismenkörper wurde zuerst von Hevesy und Hofer[1] hingewiesen. Bringt man z.B. einen kleinen Goldfisch in verdünntes schweres Wasser, dann findet ein lebhafter Austausch von H- und D-Atomen zwischen dem Inhaltswasser des Fisches und dem umgebenden schweren Wasser statt. Die Austauschgeschwindigkeit ist so gross, dass das Gleichgewicht schon innerhalb von wenigen Stunden erreicht wird. In diesem Zustand sind die D-Konzentrationen des Wassers innerhalb und ausserhalb des Fischkörpers ausgeglichen. Deshalb kann man aus der Gesamtmenge des umgebenden Wassers sowie dessen Konzentrationen an Deuterium vor und nach dem Experiment leicht den Wassergehalt des Fisches errechnen. Auf diese Weise fanden Hevesy und Hofer den Wassergehalt des lebenden Goldfisches, der sich mit dem durch Analyse ermittelten Wert als gut übereinstimmend erwies. Genau dieselben Resultate erhielt man auch, wenn man eine gegebene Menge von schwerem Wasser eingab[2] oder in den Tierkörper einspritzte.[3] Um zu sehen, ob man dasselbe Verfahren zur Ermittelung des Wassergehalts im Pflanzenkörper anwenden kann, untersuchten wir die Sojabohnen.

Die zum Versuch benutzten Bohnen waren gewöhnliche, japanische Sojabohnen, deren Durchmesser durchschnittlich 9 mm. betrug. Diese wurden an der Luft getrocknet und enthielten immer noch 10.4% Wasser. Der Wassergehalt der Bohnen wurde auf folgende Weise bestimmt: Eine abgewogene Menge Bohnen wurde zuerst sorgfältig zerkleinert und dann in einem Luftbad konstant bei 100°C. bis zum konstanten Gewicht getrocknet. Dazu waren ungefähr fünf Stunden erforderlich.

In sechs Erlenmeyerkolben brachten wir je 150 g. von nur an der Luft getrockneten Bohnen und versetzten jede Menge mit 300 c.c. von 0.14% schwerem Wasser, dessen spezifisches Gewicht 131 γ grösser als gewöhnliches Wasser war. Die Kolben wurden dann von Zeit zu Zeit mit der Hand sanft geschüttelt. Nach einer bestimmten und zwar für den einzelnen Kolben verschiedenen Zeit, wurde das Wasser von den

(1) G. von Hevesy und E. Hofer, *Z. physiol. Chem.*, **225** (1934), 28; *Nature*, **133** (1934), 495.
(2) G. von Hevesy und E. Hofer, *Nature*, **134** (1934), 879.
(3) E. J. McDougall, F. Verzar, H. Erlenmeyer und H. Gärtner, *Nature*, **134** (1934), 1006.

Bohnen abgetrennt und sorgfältig gereinigt. Die Reinigung des Wassers führten wir nach der schon oft beschriebenen Methode mittels erhitzten Kupferoxyds u.s.w. durch.[4] Das spezifische Gewicht des so gereinigten Wassers wurde dann nach der Schwebemethode mittels eines Quarzschwimmers im Vergleich zu dem des Standardwassers bestimmt. Die Messresultate gibt die folgende Tabelle wieder.

Kolben Nr.	Eintauchsdauer in Stdn.	Dichteabnahme des umgebenden Wassers in γ
1	$\frac{1}{6}$	1
2	$\frac{1}{2}$	2
3	$1\frac{1}{2}$	5
4	$4\frac{1}{2}$	10
5	20	17
6	46	16

Die letzte Reihe zeigt die Abnahme in der Dichte des abgetrennten Wassers in bezug auf dessen Anfangsdichte. Wie aus der Tabelle ersichtlich ist, ging die Austauschreaktion nicht so schnell vor sich, aber zum Schluss stellte sich das Gleichgewicht sicherlich ein. Rechnet man aus diesen Daten den Wassergehalt der Bohnen, dann ergeben sich 25%. Dieser Wert erwies sich sogar über doppelt so gross wie der durch Analyse ermittelte von 10%. Rechnet man dagegen die Dichteabnahme des umgebenden Wassers aus dem analytisch bestimmten Wassergehalt der Bohnen (10%), dann sollte sie beim Gleichgewicht 7 γ sein. Die Frage nach der Ursache dieser grossen Diskrepanz kann man natürlich nicht leicht beantworten. Aber es scheint dafür zwei Möglichkeiten zu geben. Entweder enthielten die benutzten Bohnen noch eine Menge Wasser, das durch blosse Trocknung bei 100°C. nicht entfernt werden konnte. Oder es traten nicht nur die H-Atome des Inhaltswassers der Bohnen, sondern auch die anderen austauschbaren H-Atome in den Bohnensubstanzen mit den D-Atomen des umgebenden Wassers in Austausch. Und zwar scheint die zweite Möglichkeit wahrscheinlicher als die erste. Denn bei den Versuchen von Bonhoeffer[5] und seinen Mitarbeitern wurde gefunden, dass die an O gebundenen H-Atome in der Kohlenhydraten sowie die an N gebundenen H in Eiweiss alle mit D-Atomen austauschbar sind. Und es ist kaum nötig zu bemerken, dass solche Substanzen reichlich in Bohnen vorhanden sind.

(4) Vgl. z.B. T. Titani und M. Harada, dies Bulletin, **10** (1935), 261.
(5) Vgl. z.B. K. F. Bonhoeffer, *Z. Elektrochem.*, **40** (1934), 469.

Wir sind der Hattori-Hohkohkai (Hattori-Stiftung) sowie der Gakujutsu-Shinkokai (Notgemeinschaft der japanischen Wissenschaft) für ihre finanzielle Unterstützung zum herzlichsten Dank verpflichtet.

Physikalisch-Chemisches Laboratorium der Kaiserlichen Universität zu Osaka, und Schiomi Institut für Physikalische und Chemische Forschungen.

ISOTOPENAUSTAUSCH ZWISCHEN WASSER UND EINIGEN ORGANISCHEN VERBINDUNGEN.

(Vorläufige Mitteilung.)

Von Masao HARADA und Toshizo TITANI.

Eingegangen am 19. Oktober 1935. Ausgegeben am 28. November 1935.

Eine der interessantesten Anwendungen von Deuterium liegt darin, dass die D-Atome beim Versuch über den Austausch von H-Atomen als Indikatoren verwendet werden. Wenn z.B. eine H-haltige Verbindung in schwerem Wasser gelöst, oder falls sie nicht löslich ist, damit vermischt und geschüttelt wird, findet ein Austausch von H- bzw. D-Atomen zwischen beiden Substanzen statt. Dieser Austausch wird dadurch nachgewiesen, dass die Dichte des verwendeten schweren Wassers während des Versuchs abnimmt, bis ein Gleichgewichtszustand, ein Austausch- oder Isotopengleichgewicht, erreicht wird. Infolgedessen kann man aus solch einem Versuch Schlüsse ziehen über die Austauschbarkeit von H-Atomen in der betreffenden Substanz. Bonhoeffer und seine Schüler haben auf diesem Wege zahlreiche Versuche über die Austauschreaktion zwischen Wasser und organischen Verbindungen ausgeführt.[1] Aus diesen Versuche ergab sich, dass nur solche H-Atome, die an O oder N gebunden sind, mit denen des Wassers ausgetauscht werden, aber die an C gebundenen H-Atome allgemein nicht direkt austauschbar sind. Diesen Umstand kann man eventuell dazu benutzen, um die Anzahl von Hydroxyl- oder Aminogruppen in einem gegebenen Molekül zu ermitteln.

(1) Vgl. z.B. K. F. Bonhoeffer, *Z. Elektrochem.*, **40** (1934), 469.

Aber dabei muss man, wenn quantitative Schlüsse gezogen werden sollen, auch die Verteilung von D-Atomen zwischen Wasser und verwendeten Verbindungen in Betracht ziehen, worauf Bonhoeffer schon hingewiesen hat. Denn die D-Atome zeigen oft die Neigung aus dem Wasser in die organischen Verbindungen mehr oder weniger absorbiert zu werden. Aber nicht selten gibt es auch einen entgegengesetzten Fall, wie an einem Beispiel weiter unten gezeigt wird. Diese Neigung zur Akkumulation der D-Atome kann man entweder durch den Verteilungskoeffizienten der D-Atome zwischen beiden Substanzen oder durch die Gleichgewichtskonstante der betreffenden Austauschreaktion quantitativ messen.

Falls die D-Konzentration nicht zu gross ist, kann die Austauschreaktion zwischen einem organischen Molekül RH_n, das n austauschbare H-Atome von gleicher Natur enthält, und schwerem Wasser durch die folgende Gleichung dargestellt werden.

$$RH_n + HDO \quad \rightleftarrows \quad RH_{n-1}D + H_2O,$$

wobei das D_2O-Molekül und solche wie $RH_{n-2}D_2$ u.s.f. wegen der Kleinheit der D-Konzentration vernachlässigt werden. Die Gleichgewichtskonstante K für diese Reaktion ist dann offenbar:

$$K = \frac{[RH_{n-1}D][H_2O]}{[RH_n][HDO]},$$

und der Verteilungskoeffizient k der D-Atome wird durch die folgende Formel definiert.

$$k = \frac{\text{(Atomare Konz. von D in den gesamten austauschbaren H der organischen Substanz)}}{\text{(Atomare Konz. von D in den gesamten H des Wassers.)}}.$$

Die so definierten Konstanten K und k stellen beide für eine gegebene Verbindung eine charakteristische Konstante dar, so lange wie die D-Konzentration genügend klein bleibt. Aber für den praktischen Zweck ist der Verteilungskoeffizient k anschaulicher. Denn falls $k = 1$ ist, verteilen sich die D-Atome gleichmässig zwischen dem Wasser und der organischen Verbindung, dagegen wenn $k > 1$ ist, werden sie in die organische Substanz akkumuliert und vice versa. Wir haben die oben genannten beiden Konstanten k und K für einige organische Verbindungen experimentell bestimmt. Die Ergebnisse dieses Experimentes wollen wir vorläufig mitteilen.

Durchschnittlich 2 c.c. von 1 bis 2 prozentigem schweren Wasser wurde mit einer geeigneten Menge von organischer Verbindung im

zugeschmolzenen Glasröhrchen verschiedene lange erwärmt. Dann wurde das Wasser von der organischen Verbindung auf verschiedene Wege, je nach der verwendeten Substanz, meistens bei Zimmertemperatur abgetrennt und gereinigt.[2] Die Dichte des so gereinigten Wassers wurde durch Schwebe-methode mittels eines kleinen Quarzschwimmers genau bestimmt. Die vom Wasser abgetrennte organische Substanz wurde zunächst gut getrocknet und dann im Luftstrom mittels Kupferoxyds verbrannt. Das abdestillierte Wasser wurde sorgfältig gereinigt und dessen Dichte ebenfalls genau bestimmt. Sicherheitshalber haben wir aus diesen beiden Daten sowie der Anfangsdichte des verwendeten Wassers k und K ausgerechnet, und zwar durch die Kombination von je zwei aus drei Dichtebestimmungen. Die Versuchsergebnisse sind in der folgenden Tabelle zusammengestellt, wo die festgedruckten Buchstaben **H** in der zweiten Vertikelreihe austauschbare H-Atome darstellen. Jedes dieser

Versuchsergebnisse

Substanz	Formel	Verteilungskoef. k	Gleichgewichtskonst. K
Chloroform	CH_3Cl	(kein Austausch)	
Äthyläther	$C_2H_5OC_2H_5$	(kein Austausch)	
Anilin	$C_6H_5NH_2$	1.11	1.11
Phenol	C_6H_5OH	1.07	0.54
Pyrol	C_4H_4NH	0.88	0.44
Benzaldehyd	C_6H_5CHO	(kein Austausch)	
Benzoesäure	C_6H_5COOH	1.04	0.52
Benzylalkohol	$C_6H_5CH_2OH$	1.10	0.55

Resultate ist der Durchschnitt aus zwei bis fünf unabhängig durchgeführten Messungen und die Genauigkeit dürfte durchschnittlich $\pm 5\%$ sein. Wie man leicht aus der Tabelle ersieht, ist der Verteilungskoeffizient k in jedem Fall nicht viel von eins verschieden, mindestens unter den vorliegenden Versuchsbedingungen.

Zum Schluss möchten wir Herrn Dr. Morita in unserem Laboratorium, der uns einen Teil des verwendeten schweren Wassers zur Verfügung gestellt hat, besten Dank aussprechen. Zugleich ist es unsere angenehme Pflicht der Hattori Hohkohkai (Hattori-Stiftung) sowie der

(2) Die ausführliche Publikation über die Arbeitsmethode wird später in diesem Bulletin erfolgen.

Gakujutsu-Shinkokai (Notgemeinschaft der japanischen Wissenschaft) für ihre finanzielle Unterstützung unseren herzlichsten Dank auszusprechen.

Schiomi Institut für Physikalische und Chemische Forschungen, und Physikalisch-Chemisches Laboratorium der Kaiserlichen Universität zu Osaka.

MONODEUTERIOBENZOL.

Von **Noriyoshi MORITA** und **Toshizo TITANI.**

Eingegangen am 2. November 1935. Ausgegeben am 28. November 1935.

Hinsichtlich des Benzolproblems hat die Untersuchung über die Deuteriobenzole so grosses Interesse hervorgerufen, dass schon viele Versuche über deren Darstellungsmethoden veröffentlicht wurden. Aber in manchen Fällen handelt es sich um das total substituierte Hexadeuteriobenzol C_6D_6, oder ein Gemisch aus teilweise substituierten verschiedenen Deuteriobenzolen. Nur selten kam in die Reindarstellung von einzelnen Deuteriobenzolen in Frage. Ingold und seine Mitarbeiter wendeten die Grignardreaktion zur Darstellung von reinem Monodeuteriobenzol an.[1] Wir haben zu demselben Zweck die Doppelumsetzung zwischen Calciumbenzoat und Calciumhydroxyd benutzt. Diese Reaktion ist schon früher als Darstellungsmethode von reinem Benzol allgemein verwendet worden. In der vorliegenden Arbeit haben wir natürlich Calciumhydrat durch Calciumdeuterat ersetzt:

$$(C_6H_5COO)_2Ca + Ca(OD)_2 = 2C_6H_5D + 2CaCO_3 .$$

In der Praxis sind wir folgendermassen verfahren: 15 g. Calciumbenzoatdihydrat $Ca(C_7H_6O_2)_2{\cdot}2H_2O$ wurden bei 150°C. im Vacuum gut entwässert. Das Calciumdeuterat $Ca(OD)_2$ wurde aus 7.5 g. Calciumoxyd CaO, das im voraus bei 500°C. im Vakuum getrocknet war, und 1 g. 97% D_2O gewonnen. Das so hergestellte Calciumbenzoat und Calcium-

(1) W. R. Angus, A. H. Leckie, C. R. Bailey, C. G. Raisin, J. L. Gleave, C. L. Wilson und C. K. Ingold, *Nature*, **135** (1935), 1033.

deuterat wurden miteinander gut vermischt und im elektrischen Ofen bis auf ca. 300°C. erhitzt. Das entstehende und abdestillierte Monodeuteriobenzol wurde durch die Abkühlung mit kaltem Wasser kondensiert. Das erhaltene Produkt wurde mit Phosphorpentoxyd getrocknet und durch paarmalige Destillationen gereinigt. Die Ausbeute war 5 g. gegenüber der theoretischen von 8 g.

Nach derselben Methode, aber unter Benutzung von gewöhnlichem Wasser, haben wir auch gewöhnliches Benzol hergestellt und einige von dessen physikalischen Eigenschaften mit denen des Monodeuteriobenzols verglichen. Das spezifische Gewicht von C_6H_6 betrug $d_{25}^{25} = 0.8754$, während das des 97 prozentigen C_6H_5D $d_{25}^{25} = 0.8869$ war. Der erste Wert stimmt sehr gut mit dem Standardwert für reines C_6H_6 überein. Dies kann als ein Beweis für die Reinheit des von uns hergestellten C_6H_6 sowie C_6H_5D betrachtet werden. Angenommen, C_6H_5D besäße dasselbe Molekularvolumen wie C_6H_6, dann sollte das spezifisches Gewicht von 97% C_6H_5D $d_{25}^{25} = 0.8864$ sein. Dieser Wert erwies sich als gut übereinstimmend mit dem gefundenen. Der Schmelzpunkt von C_6H_5D liegt um etwa 0.1°C. höher als der des C_6H_6. Zum Schluss haben wir das Brechungsvermögen von beiden Benzolen mittels eines Interferometers verglichen. Dabei ergab sich, dass die Brechungszahl von C_6H_5D für weisses Licht bei 23°C. um 0.00065 kleiner als die des C_6H_6 ist.

Die oben beschriebene Methode gestattet uns durch die verhältnismässig einfache Manipulation ein reines Produkt zu erhalten. Aber ein anderer Vorteil der Methode liegt darin, dass man alle Deuteriumatome im verwendeten schweren Wasser zur Substitution benutzen kann. Nach demselben Prinzip kann man auch andere verschiedenen Deuteriobenzole rein herstellen. Solche Versuche sind jetzt im Gang.

Zum Schluss ist es unsere angenehme Pflicht der Gakujutsu-Shinkokai (der Notgemeinschaft der japanischen Wissenschaft) sowie der Hattori-Hohkohkai (der Hattori-Stiftung) für ihre finanzielle Unterstützung unseren herzlichsten Dank auszusprechen.

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